Phylogeography of the *Cellana* limpets of New Zealand: Investigating Barriers to Marine Dispersal and Historical Biogeography

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Abstract

New Zealand is a continental island surrounded by deep-ocean, with a complex system of currents and oceanographic anomalies that may serve to isolate populations of coastal marine organisms. In particular Cook Strait is a 26 km wide body of water separating the North and South Islands which is characterised by complex interactions of tides and converging water masses. Cook Strait is a geologically recent formation that may also impart an historical influence on the biogeographic distribution of coastal marine taxa. In order to investigate the phylogeographic structure of coastal marine taxa around New Zealand's coastline and to investigate the processes that may contribute toward this genetic structure, I analysed mitochondrial DNA genes from limpet species of the *Cellana* genus.

Phylogenetic analyses of the ribosomal 12S and 16S DNA genes showed that *C. ornata* may approximate the founding lineage to New Zealand, but these genes were unable to resolve between contrasting biogeographic hypotheses about the origin of *Cellana* in New Zealand. Intraspecific analyses of the mitochondrial cytochrome *b* gene from populations throughout the biogeographic range of *Cellana ornata*, *C. radians* and *C. flava* showed concordant genetic discontinuity at Cook Strait. Further analyses suggest that allopatric fragmentation and restricted gene flow have caused significant genetic differentiation between populations of the North and South Island. Demographic expansion was also identified for *C. radians*. The influence of contemporary nearshore currents and sea surface temperature was assessed using the mitochondrial cytochrome b gene for *C. radians* and *C. ornata*. Low levels of genetic differentiation between populations corresponding to 'current-zones' suggests that contemporary oceanic processes may be contributing to the genetic pattern observed for these species. However, the shallow divergence between haplotypes and populations restricted thorough investigation of contemporary gene flow. Genealogical concordance across co-distributed marine taxa of New Zealand also lends support to the historic association of genetic and species discontinuities with recent geological changes, such as sea level fluctuations.

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Chapter I

General Introduction

"Every naturalist who has directed his attention to the subject of geographical distribution of animals and plants, must have been interested in the singular facts which it presents…..Of late years, … a great light has been thrown upon the subject by geological investigations, which have shown that the present state of the earth, and the organisms now inhabiting it, are but the last stage of a long and uninterrupted series of changes which it has undergone, and consequently, that to endeavour to explain and account for its present condition without any reference to those changes (as has frequently been done) must lead to very imperfect and erroneous conclusions"

- Alfred Russel Wallace (1855)

1.1 Phylogeography

Avise (1987) introduced the term phylogeography to include the integration of phylogenetics and the geographical distribution of genetic variation. This integration has increased the resolution for interpretation of evolutionary pattern and process. The examination of phylogeography and gene flow in the marine environment has increased considerably over the last two decades. There are now numerous studies outlining the genetic pattern and geographic association of speciation and population structuring in marine environments from the open ocean and deep abyss (Craddock et al., 1997; Distel et al., 2000; Smith et al., 2004) to rocky platforms (De Wolf et al., 2000; Lessios et al., 2001) and soft benthos (Backeljau et al., 1994; Benzie and Williams, 1998; Lambert et al., 2003) of coastal shores.

Phylogeography has evolved from and still includes the molecular investigation of contemporary adult populations to determine the patterns and processes behind speciation and population connectivity or gene flow (Avise, 2004). The advancement of molecular techniques and the recognition of evolutionary theory and geographic influence have provided means of testing evolutionary and ecological hypotheses of population connectivity and gene flow (Purugganan and Gibson, 2003).

Many molecular studies are primarily concerned with the demographic effects of life history variation. These studies focus on comparative examinations involving species of varying dispersal potential. For instance, Goldson et al. (2001) examined the genetic population structure of two bryozoans (*Celleporella hyaline* and *Electra pilosa*) with contrasting modes of larval development from the Menai Strait, North Wales. They found that genetic structure was consistent with the larval modes and local hydrography. *C. hyaline*; a brooding species, exhibited genetic differentiation over 10 metres, while *E. pilosa*, with a planktotrophic larval stage only showed high levels of genetic heterogeneity over 70km, at which point oceanic conditions appeared to restrict gene flow for this species.

Other studies tend toward discerning between putative dispersal barriers and historical influence as determinants in the genetic structuring of populations. For example, Marko (2004) used two gastropods, *Nucella ostrina* and *N. lamellose*, both lacking a planktonic larval stage, to test contrasting hypotheses of dispersal and survival in refugia during the Last Glacial Maximum in British Columbia and Alaska. The

results differed for each species. A demographic bottleneck and subsequent expansion was inferred for *N. ostrina* and post-glacial colonisation with isolation-by-distance was inferred for *N. lamellose* (Marko, 2004).

1.2 Dispersal

Population connectivity is maintained through migration (Wright, 1969). However, most coastal marine organisms have a sedentary or semi-sessile adult life stage limiting their migration. Consequently, marine invertebrates with a limited capacity for dispersal as adults rely on dispersive larval stages (Hedgecock, 1986). Larval stages can be categorised based on the mode of larval nutrition and the duration of the planktonic phase (Thorson, 1950). Thorson's (1950) categories of larval mode included planktonic feeding larvae (planktotrophic) and non-feeding larvae (lecithotrophic). A more descriptive larval classification was introduced by Scheltema (1971) for the purpose of studying dispersal potential. The terms teleplanic, actaeplanic, anchiplanic and aplanic were used to complement Thorson's earlier classifications. Planktotrophic larvae are generally classed as teleplanic, spending greater than 2 months in the water column, or actaeplanic, with a period of 1 week to 2 months in the plankton. Lecithotrophic larvae are more likely to spend only hours or days (anchiplanic) in the plankton or not enter the water column at all (aplanic) in the case of brooders (Scheltema, 1971, 1988; Boidron-Métairon, 1995). The revised larval classification is important in determining the influence of the oceanic environment on the dispersive life-history stage of marine organisms.

For those organisms with an extended planktonic larval stage, coastal and oceanic waters influence the colonization of new environments (Ladd, 1960; Scheltema and Williams, 1983). Although active settlement, delayed metamorphosis and vertical migration are well known larval traits (Mileikovsky, 1973), a fresh interest in larval behaviour has seen the introduction of a new field termed "supply-side ecology" (Lewin, 1987). This field combines larval behaviour and larval distributions with the processes acting on larvae in the water column, and in turn, their influence on the abundance and distribution of juveniles and adults (Carlon, 2002).

The microscopic nature of larval stages makes field observations difficult for most species (Levin, 1990), although larvae of some organisms such as coral (Carlon

and Olson, 1993) can be tracked by divers. Laboratory-reared larvae have been used instead to test factors that may influence dispersal. For example, laboratory experiments have tested the effects of age-related behaviour (Raimondi and Keough, 1990; Barile et al., 1994), settlement and metamorphic cues (Pawlik and Hadfield, 1990; Pechenik and Gee, 1993; Roberts and Lapworth, 2001) and larval mortality (Jackson and Strathmann, 1981; Johnson and Shanks, 2003) on dispersal potential.

Collection of larvae from the water column has also contributed to the long debated mechanism of larval transport (Butman, 1989): are larvae transported passively by the ocean currents, or do they actively control their position within the water?. Larval distribution and oceanographic influence within the water column have been investigated through field collection of larvae using modified sediment traps (Butman, 1989; Castilla, 2001), net tows (Miller and Emlet, 1997; Stoner and Smith, 1998) and filter pump systems (Banse, 1986; Graham and Sebens, 1996). Using these methods, processes such as internal tidal bore warm fronts (Pineda, 1999) and wind-induced, across-shelf transport (Stoner and Smith, 1998) have been shown to influence the distribution of barnacle and gastropod larvae within the water column, and therefore their potential to disperse.

Dispersal potential estimated using laboratory experiments and field sampling is not necessarily the best indicator of realised dispersal leading to gene flow. However, in combination with the distribution of genetic variation within and between populations, an indirect measure of dispersal leading to gene flow can be more accurately estimated. This indirect measure has been used extensively in the marine environment to show the influence of life history on population connectivity and gene flow (Hellberg, 1996; Ayre et al., 1997; Perrin, 2002).

Organisms with contrasting life histories are expected to show different levels of genetic structure (Ayre et al. 1997). Species with low dispersal potential, such as those with an anchiplanic or aplanic larval phase, have been shown to exhibit greater genetic structure between populations than species with teleplanic or actaeplanic larvae (Lambert et al., 2003). For example, siphonariid limpets capable of long distance dispersal, exhibited low levels of genetic differentiation between islands in the Houtman Abrolohos Islands,Western Australia (Johnson et al., 2001). However, there are instances where a high degree of genetic structuring between populations cannot be

explained by dispersal potential (Johnson and Black, 1982; Hedgecock, 1986; Kyle and Boulding, 2000; Barber et al., 2002). For example, the nudibranch, *Adalaria proxima*, exhibited greater genetic structuring over a distance of less than 10 km than was expected from its moderate dispersal potential (Todd et al., 1998). Genetic structuring such as this, could be an indication that larval dispersal is not an important factor in the maintenance of gene flow (Hedgecock, 1986), and that post-larval life phases are driving the genetic structuring of populations; or that dispersal is being altered or restricted by an external factor such as a physical or chemical barrier or demographic disparity between populations.

1.3 Dispersal Barriers

Allopatric speciation results from the reproductive isolation of populations. Isolation can originate from physical barriers separating the populations and restricting gene flow long enough for genetic drift to cause divergence between them (Hellberg, 1998). If the "barrier" is substantial in time and space and restricts all migration or dispersal, then allopatric speciation will occur. This infers that barriers can be recent or historical in nature. Global scale isolation, leading to speciation, has occurred among taxa now exhibiting an anti-tropical distribution, as seen in the patellid limpets (Lindberg, 1991; Koufopanou et al., 1999), and taxa confined by large ocean basins, including the Arctic Ocean and the North and East Pacific Ocean (Hellberg, 1998). If a barrier is recent, leaky or ephemeral in nature, then population sub-division can be observed (Avise, 2000).

The level of isolation among populations can be determined by analysis of genetic structure between populations to infer process from the distribution of genetic variation observed. This approach has been widely used to identify dispersal barriers for marine populations (Benzie and Williams, 1997). For example, the giant clam *Tridacna maxima*, ranging throughout the Indo-West Pacific, exhibited genetic structuring consistent with historical fluctuation in sea levels (Benzie and Williams, 1997). Similarly, the fucalean seaweed *Fucus serratus* was shown to exhibit expansion from refugial populations after the last glacial maximum in northern Europe (Coyer et al., 2003). On a more ecological time scale, a sandy expanse at Santa Monica Bay, USA was shown to restrict dispersal between populations of the reef fish *Embiotoca*

jacksoni (Bernardi, 2000) and at a finer scale, habitat heterogeneity and island isolation within the Koster archipelago along the Swedish west coast, affected the genetic structuring of the marine snail *Littorina saxatilis* (Johanesson et al., 2004).

The emergence of the Florida Peninsula and the Isthmus of Panama are two well-known land barriers isolating marine populations (Jackson et al., 1993; Knowlton et al., 1993; Lessios et al., 1999). Other marine barriers are not as visible as land formation and direct observation of their effects on the dispersal of particular species is difficult or impossible. Current examples of known marine barriers include upwelling zones such as those observed around Point Conception, USA (Miller and Emlet, 1997; Connolly et al., 2001; Wares et al., 2001), directional currents such as tidal fronts and along-shore flows (Farrell et al., 1991; Richards et al., 1995; Pineda, 1999; Rios et al., 2002), and substratum and temperature and salinity gradients as seen along contiguous shore lines (Gaylord and Gaines, 2000; Pineda et al., 2002).

Concordance of phylogeographic structure and geographic discontinuities is an important, but still indirect, step in determining the influence of historical and contemporary isolating barriers (Taberlet et al., 1998; Avise, 2000; Dawson, 2001; Wares and Cunningham, 2001; Hurtado et al., 2004). For example, Dawson (2001) compared the genetic structure across a variety of marine taxa to examine the biogeographic discontinuity proposed by separate biogeographic and phylogeographic studies around Point Conception, USA (Dawson, 2001). Point Conception is a region at which upwelling events and directional currents have been reported to cause both species and population discontinuities (Gaylord and Gaines, 2000). Dawson (2001) found that taxa with greater dispersal ability generally had less phylogeographic structure, although many exhibited phylogeographic breaks within the California Transition Zone identified by earlier studies. Phylogeographic gaps, edge-effect species, and ecotones were found to coincide with Late Pleistocene faunal discontinuities and probable long-term physical barriers to gene flow. Many other illustrative examples of concordance have been extensively reviewed by Avise (Avise, 2000, 2004).

1.4 Demographic Disparity

The study of demography includes age-specific distributions of survival, growth, recruitment and reproduction (Hartl and Clark, 1989). Demographic disparity between populations could cause population structuring that is erroneously inferred to be the result of dispersal or vicariance. For example, bottleneck and founder events reduce the number of reproductive individuals contributing to the genetic variation of a population (effective population size, *Ne*) (Harding, 1996). Fluctuation in the effective population size creates inconsistencies in the rate at which lineages coalesce (ie. share a common ancestor) to the most recent common ancestor shared by all populations (Harding, 1996). A decrease in the effective population size increases the rate of coalescence, reducing the perceived time to the most recent common ancestor by increasing the number of recent genetic mutations within a population (DeSalle and Templeton, 1988; Galtier et al., 2000).

The most commonly cited historical event to isolate populations is the last glacial maximum. During this time many species underwent a bottleneck period as sea temperature and sea level fluctuations caused northward or southward retreat, and subsequent recolonisation of denuded areas from refugial populations (Lloyd, 2003; Coyer, 2003, Jolly, 2004). For example, many European taxa, including mammals, amphibians, arthropods and plants have been shown to exhibit signs of southward retreat with subsequent northward colonisation resulting from the Quaternary cold periods in Europe (Taberlet et al., 1998).

Spawning time and fecundity asymmetries can also cause disparity in the rate of evolution between populations and create misleading genetic structure. The physical conditions at the time of larval release or spawning establish the initial conditions of larval survival, trait expression and dispersal direction (Sponaugle et al., 2002; Yamahira, 2004; Hendry and Day, 2005). Therefore, gene flow between populations may be restricted due to dispersal direction and larval survival variation based on the timing of release. Hendry and Day (2005) have called this “isolation-by-time” as an analogy to isolation-by-distance. Isolation-by-time has been shown through modeling of larval transport off the east coast of New Zealand (Stephens et al., 2004). Here, particle release was modeled with different wind direction as measured at different times of the year. They showed that wind direction determined the distance and

direction of particle transport along the coast from a common release point, suggesting that release time is influential in predicting the direction and distance of larvae due to prevailing wind conditions.

Spawning cues can be very specific (Hendry and Day, 2005) and may be directly related to seasonal conditions. For instance, Grange (1976) found that vigorous water motion stimulated spawning in some trochid and turbinid snails, *Melagraphia aethiops*, *Zediloma atrovierens* and *Lunella (Turbo) smaragda*. Similarly spawning by the limpet *Lottia digitalis* appears to be triggered by rough seas (Shanks, 1998), while other taxa such as crabs and fish have been shown to respond to tidal amplitude and moon-phase (Morgan, 1996; Sponaugle and Pinkard, 2004).

Demographic disparity can also occur where adult fecundity varies between populations. Variation in adult fecundity is largely dependent on the surrounding environment and the stresses it imparts on the individual (Chaparro, 1990), which is in turn directly related to reproductive investment. Two populations that are not experiencing the same conditions can show disparity in paternal investment and fecundity, and therefore effective population size and genetic variation. For instance, larger individuals tend to have a higher reproductive output (Dunmore and Schiel, 2000) and adult size can be influenced by nutrition (Chaparro, 1990), degree of wave exposure (Brown and Quinn, 1988; Hobday, 1995), parasitism (gigantism) and population density (Curtis, 1995), all of which can vary between populations.

Despite the high prevalence of parasitism in intertidal organisms it is often overlooked as a factor in population and ecological studies. When parasitism reaches the limit to which the host is adapted, parasitized individuals often display reduced survival and fecundity (Donald et al., 2004), with extreme cases resulting in parasitic castration (Jokela and Lively, 1995). The level of parasitism often varies between individuals within a population and certainly between populations (Jokela and Lively, 1995).

Differential mortality of larval and juvenile life-stages will also effect the perceived genetic variation between populations (Haag and Garton, 1995). Gardner and Kathiravetpillai (1997) used the leucine aminopeptidase (LAP) locus to show a correlation between genetic variation and water salinity in the mussel *Perna canaliculus*. They found that increased salinity caused a frequency shift of alleles to

occur in juvenile samples. Schneider et al. (2003) showed that increased larval mortality at the D-stage (veliger) of zebra mussel larvae caused spatial variation in adult population density downstream in the Ilinois River, illustrating how differential mortality can contribute to disparity in the population size between sites.

1.5 Phylogeography in New Zealand

One of the earliest population genetic studies in New Zealand was by Smith (1980) who used allozyme techniques on the rock lobsters *Jasus edwarsii* and *Jasus novaehollandiae*. Although there are still relatively few studies of marine phylogeography in New Zealand, considerable progress has been made in the use of genetic tools to investigate population structuring, speciation and the influence of New Zealand's well characterised palaeo- and contemporary oceanographic characteristics (Smith et al., 1989; Intasuwan et al., 1993; Mladenov et al., 1997; Triantafillos and Adams, 2001; Apte and Gardner, 2002; Perrin, 2002; Sponer and Roy, 2002; Smith et al., 2004; Waters and Roy, 2004). A detailed review of these studies is given in Chapter Five.

In general, New Zealand studies have focused mostly on the population genetic structure of commercial fisheries stocks. Fisheries zones or collection sites have been used in these studies to show connectivity between stocks (Smith, 1980; Smith et al., 1986; Ovenden, 1992; Smolenski, 1993; Smith, 1996). More recently, phylogeographic hypotheses have been tested across perceived dispersal barriers (Sponer and Roy, 2002; Star et al., 2003; Waters and Roy, 2004; Stevens and Hogg, 2004). These studies have examined the population genetic structure of coastal invertebrates with respect to biogeographic discontinuities, nearshore oceanographic processes and historical vicariance. A conclusion shared among these studies is that populations north and south of Cook Strait are partially separated. Stevens and Hogg (2004) provided evidence for allopatric fragmentation of two corophiid amphipod species distributed across Cook Strait, while other studies report the influence of upwelling events and nearshore currents as processes structuring the populations in this region (Apte and Gardner, 2002; Star et al., 2003; Waters and Roy, 2004).

1.6 Historic New Zealand

New Zealand has been characterised as a continental island comprising 600 islands that extend from the sub-tropical to sub-Antarctic zones of the Southwest Pacific and cover 268,000km^2 (Daugherty et al., 1993; Towns and Ballantine, 1993). It is a remnant of a larger land mass known as the New Zealand geosyncline, which first formed at the margin of Gondwana and included New Caledonia, Lord Howe Rise, Norfolk Ridge, Chatham Rise and the Campbell Plateau (Fleming, 1979; Stevens, 1980). Well-preserved fossil and geological records have allowed the climatic and geological changes of New Zealand to be inferred, although there are still many areas of debate.

The coastline has changed considerably since the separation from Gondwana and Australia 80-60 million years ago (mya). The geosyncline was a peneplain land mass experiencing oceanic conditions (Fleming, 1979; Stevens, 1980; Stevens et al., 1995) until the onset of sea-level fluctuations throughout the Cenozoic generated transient island formation (Cooper and Millener, 1993). During the Oligocene marine transgression, the New Zealand land mass was reduced to just 18% of its present area (Fleming, 1979; Stevens, 1980; Cooper and Millener, 1993). At the same time volcanic activity occurred around East Cape, Canterbury, Marlborough and Oamaru, while the North Island was partly submerged in bathyal conditions and underwent volcanistic phases throughout the Oligocene and mid Miocene (Stevens, 1980). Offshore, continental islands formed as marine transgression left some areas of the Chatham Islands with shallow seas, and other parts of the Chatham Rise, Campbell Plateau and Lord Howe Rise submerged in deep sea, separating these islands from New Zealand approximately 70 mya (Fleming, 1979; Stilwell, 1997).

The onset of the Kaikoura orogeny during the Pliocene mountain building period (5-2 mya) and Pleistocene sea level changes (2 my-5000 ya) once again altered the coastal environment. The alternating rise and fall of the sea level during this period saw the intertidal zone lowered to 150-200 m below present levels. Large fluctuations occurred during transitions between glacial and interglacial periods with a final rapid rise of 1m per 100 yrs to the present level 10,000 – 5000 ya (Fleming, 1979).

Historical climatic conditions surrounding New Zealand are continually revised as geologists find new techniques to study the ocean bed, with a concentration of research relating to the last one million years (Hayward et al., 2002; Hayward et al., 2003; Marra, 2003; Mildenhall, 2003). After its separation from Gondwana (80-60 mya) New Zealand was mainly situated within the Antarctic Circle with cool temperate climatic conditions (Fleming, 1979). Temperatures gradually warmed with the northward migration of the land mass during the Cenozoic, up to the Miocene thermal maximum 25 mya. West Antarctic glaciation and the circumpolar current brought cool water back to New Zealand approximately 15 and 5 mya (Beu and Maxwell, 1990). Sea surface temperature during the glacials was tempered by the onset of the Tasman current, at least on the West coast, and unlike the terrestrial environment the coastal water did not reach glacial temperatures (Knox, 1980; Nelson et al., 1993a). There was, however, a large drop in temperature in the south east of New Zealand around 18,000 ya with an increase in current intensity and a northward migration of the Antarctic and subtropical convergence zones (Moore et al., 1980; Nelson et al.). Temperature gradients on the South Island, west to east and north to south, throughout the Cenozoic, were steeper than present but maximum temperatures were not as warm as present sea surface temperatures (Heath, 1982).

1.7 New Zealand's Present Oceanic Conditions

The present sea surface currents surrounding New Zealand have been well characterised (Heath, 1985; Vincent et al., 1991; Uddstrom and Oien, 1999). There are six major nearshore currents circulating around New Zealand's coastal waters (Fig.1.1). These currents produce strong gradients in temperature and salinity along the South Island, both from north to south and east to west (Vincent et al., 1991; Uddstrom and Oien, 1999). The East Auckland Current (EAC) flowing southeast along the north eastern coast originates in the Tasman Sea and transports warm tropical water onto New Zealand's coastline. The West Auckland Current (WAC) flowing south as an offshoot of the EAC potentially transports tropical biota down the west coast and isolates the north-eastern region. Variable flow direction is seen on the east coast of the North Island with the southward flowing East Cape Current (EC) dominant in the region but accompanied by onshore northward flowing currents. On the South Island the dominant

flow is from the Southland Current (SC) which mixes sub-tropical water from the Tasman Sea with colder Sub-Antarctic water as it flows south down the west coast and north along the east coast. At Banks Peninsula this current partially deflects out to the Chatham Rise while an offshoot continues north past Banks Peninsula to Kaikoura and Cape Campbell where it again heads offshore or into Cook Strait, depending on the prevailing wind conditions (Heath, 1972). On the west coast the northward flowing Westland Current (WC) dominates during periods of consistent onshore winds (Shirtcliffe et al., 1990). This current heads north into Cook Strait where it mixes with the D'urville current originating from the South Taranaki Bight. New Zealand also bisects the subtropical Convergence (STC) which marks the merging of subtropical and sub-Antarctic waters (Gall et al., 1999).

The most complex conditions of New Zealand's coastal waters occur between the North and South Island in Cook Strait (Fig 1.1). The complexity of Cook Strait is due to the merging of water masses (Murdoch et al., 1990). Cook Strait is a point of convergence for the D'urville Current from the west, the Southland Current sweeping in from the southeast, and the southward flowing East Cape Current from the northeast. Together these currents produce a net northwest flow (Heath, 1985) and a sharp temperature interface with steep vertical gradients and irregular eddy formation (Vincent et al., 1991).

The complexity of the New Zealand coastal currents is enhanced by the many eddies, wind-induced upwelling events, fronts, gyres, and freshwater plumes from rivers (Vincent et al., 1991). Wind plays a major role in determining the nature of the currents (Heath, 1972; Shirtcliffe, et al., 1990). Strong winds are known to induce upwelling in the coastal regions. Northerly winds induce upwelling around the Kaikoura Peninsula (Heath, 1972; Chiswell and Schiel, 2001), while Southerly winds cause a drop in temperature due to the acceleration of the Southland Current transporting colder sub-Antarctic water to the north (Chiswell and Schiel, 2001). There is also evidence for the mixing of subtropical and sub-Antarctic water around this region which is possibly due to the intrusion of East Cape Current offshoots into the Southland Current flow (Uddstrom and Oien, 1999; Murphy et al., 2001). Along-shore flow and upwelling induced by south-westerly winds on the west coast of the South Island, originating near Kahurangi point to Cape Foulwind, have been observed by oceanographic cruises

during periods from October 1951, Jan/Feb 1980, March/April 1983 and January 1984 (Shirtcliffe et al., 1990) and late 1982 to February 1983 (Greig et al., 1988). However, there is ongoing debate as to whether the observed temperature changes are due to upwelling, river input or rainfall (see review by Schiel, 2004).

Major eddies have also been characterised around the coastline (Fig. 1.1). Retention of oceanic species of zooplankton and eggs of benthic and nearshore organisms has been observed in a large eddy around Blueskin Bay, north of the Otago Peninsula (Jillet, 1969; Murdoch, 1989). Similarly, the Wairarapa Eddy is a large, permanent eddy off the east coast of the North Island which has also been shown to retain larvae of the rock lobster, *Jasus edwarsii* (Chiswell and Roemmich, 1998; Chiswell and Booth, 1999) .

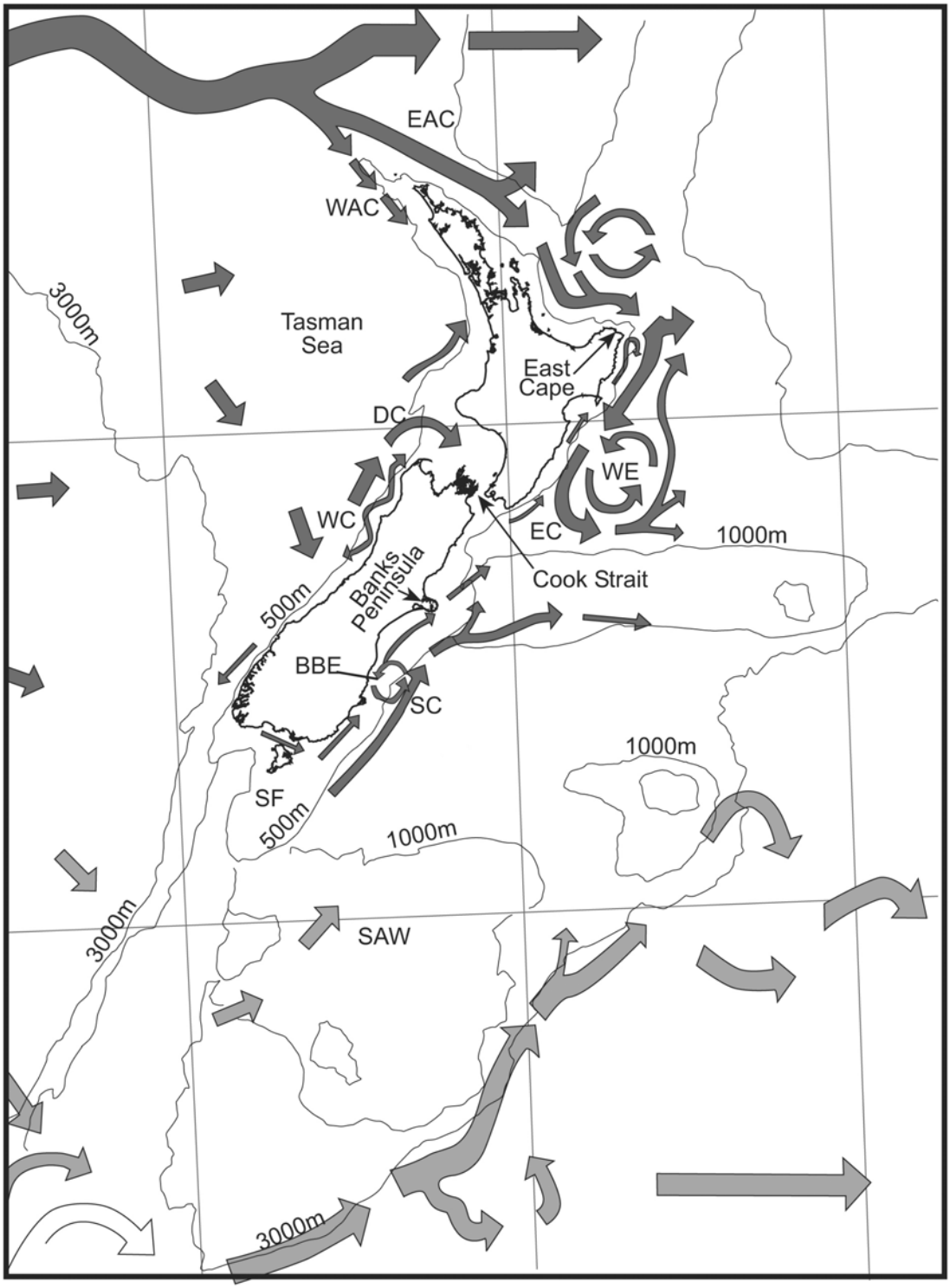

Figure 1.1. Sea surface currents and continental shelf area around New Zealand. Abbreviations: East Auckland Current (EAC); West Auckland Current (WAC); East Cape Current (EC); Southland Current (SC); Westland Current (WC); D'urville Current (DC); Wairarapa Eddy (WE); Blue Skin Bay Eddy (BBE). Redrawn from a map by National Institute of Water and Atmospheric Research.

1.8 Colonisation of New Zealand's intertidal zone

The composition and biogeographic provinces of the coastal marine fauna and flora of New Zealand have been described and reviewed on several occasions (Dell, 1961; Pawson, 1961; Creese, 1988; Nelson, 1994; Schiel, 2004), although Morton and Miller (1973) is still the definitive guide to New Zealand's seashores. The intertidal rocky shores are dominated by mussel and barnacles on the west coast, while the east coast is dominated by large brown algae and gastropod grazers, such as limpets and trochid snails (Schiel, 2004). Although rocky platforms dominate much of the coastline, they present a patchy network of varied substratum type for marine biota and contribute to the biogeographic boundaries of the coastal species (Creese, 1988).

The origin and regional influence of coastal taxa have been assessed with respect to present and historical distribution and composition of the coastal environment. New Zealand has been surrounded by deep oceans and isolated from the nearest land mass by distances greater than 1000km since the opening of the Fiji Basin 40 mya (Cooper and Millener, 1993). Fossil records show an increase in molluscan diversity around New Zealand through the late Cretaceous and Cenozoic either from original Gondwanan fauna or through immigration from elsewhere (Beu and Maxwell, 1990). Sea level changes throughout the Cenozoic are also thought to have encouraged speciation and endemism within New Zealand through successive invasions and isolations between islands (Fleming, 1979; Beu and Maxwell, 1990).

Tethyan influence and oceanic links with Austral (Antarctic to sub-Antarctic) fauna are evident during the Cretaceous, with tropical and subtropical immigrants appearing in the north throughout the Tertiary (Fleming, 1979; Stevens, 1980; Stilwell, 1997, 2003). Ephemeral island formation occurred throughout Australasia during the Palaeocene and up to 30 mya (Stevens, 1980). An island arc, including the Kermadec and Colville Ridges, might also have provided an island-hopping chain to the Three Kings Rise of New Zealand up to 40 mya, before the opening of the Fiji Basin (Cooper and Millener, 1993), permitting the ongoing immigration of Australasian and Malayo-Pacific warm-water fauna into the Oligocene (Fleming, 1979).

The final separation between Australia and Antarctica formed the Southern Ocean approximately 40 mya which, in conjunction with the opening of the Drake Passage c. 25 mya, established the circumpolar current (Stevens et al., 1995). The

circumpolar current and resulting west-wind drift is thought to have facilitated the eastward dispersal of organisms since 30 mya (Beu and Maxwell, 1990). This current also initiated the mid Miocene cooling, creating a strong sub-Antarctic faunal element, with the extinction of sub-tropical and tropical taxa in New Zealand (Fleming, 1979; Stevens, 1980; Beu and Maxwell, 1990; Cooper and Millener, 1993; Daugherty et al., 1993).

Tertiary fauna were partly retained into the Pliocene with the addition of Australian, Pacific or Austral immigrants until 30,000 ya during the glacial periods of the Pleistocene when the sub-Antarctic fauna expanded north (Fleming, 1979).

The Antarctic convergence zone, formed during the Eocene, initiated the sub-tropical convergence zone and created a permanent, steep, temperature gradient south of Campbell Island. The northward movement of the Antarctic and subtropical convergence zones is thought to have occurred several times and caused a northward range expansion of southern taxa into New Zealand waters (Knox, 1980). This expansion may have ended with the establishment of the Tasman current in oceanic circulation around New Zealand, after which southward range expansions would have been predominant.

1.9 *Cellana*

Although fossil evidence is sparse for intertidal molluscs, limpets of the genus *Cellana* are thought to have appeared in New Zealand during the Eocene. Fossils from the Paparoa Trough within the Omotumotu member have been tentatively assigned to the *Cellana* genus based on the similarity in shell characteristics to present limpets, particularly *C. denticulata* and *C. strigilis*. Shell microstructure analysis is now the accepted method of fossil identification (Lindberg and Hickman, 1986), so the shells from the Omotumotu member are only tentatively assigned to the *Cellana* genus.

Six species of *Cellana*, all of which are endemic to New Zealand, occur on the New Zealand mainland and offshore islands (Fig 1.2) and sub-species of one taxon (*Cellana strigilis)* are also found on the New Zealand and Australian sub-Antarctic islands and Chatham Island (Powell, 1955, 1973, 1979). The 32 species of *Cellana* exhibit a mostly tropical distribution, extending north to Japan, East to Juan Fernandez and Hawaii, south to South Africa, Madagascar, south east Australia, New Zealand and

the sub-Antarctic Islands, and the genus co-exists on Campbell Island with the sister taxon *Nacella* (Powell, 1973). *Nacella* species are restricted to the sub-Antarctic and Magellanic region (Powell, 1973).

Shell form, ornamentation and colour are the main taxonomic characteristics used in the identification of limpet species (Thomas, 1919; Powell, 1979). When available, the soft-tissue coloration is also used, including the head, foot and gonad, and the radula teeth can often distinguish ambiguous species (Powell, 1973). Within New Zealand there is a large range of shell morphologies both within and between species. *C. ornata* exhibits the most uniform shell morphology (Fig. 1.2), although even in this species there is considerable variation with size (Dunmore and Schiel, 2000). Morphological ambiguity exists between species which are often difficult to distinguish at first inspection. *C. flava* is most readily identified due to its plain orange shell coloration (Fig. 1.2) and light coloured head and foot, although *C. radians* has a similar morphotype occurring around Castle Point, which at first glance would be mistaken for *C. flava,* except that the soft-tissues are darker in colour (Pers. Obs.). *C. radians* is the most morphologically diverse species, exhibiting many different morphs within a single location (Fig. 1.2). This morphological diversity originally had the different morphologies classified as sub-species in the 1800-1900s, until Thomas (1919) merged the five sub-species to reassign only two morphotypes (perana and earli). *C. denticulata* and *C. strigilis* are very similar in shell colour and ornamentation except that the denticulated ridges are more pronounced for *C. denticulata* (Fig. 1.2) and the female gonads of *C. strigilis* are pink in colour (Pers. Obs.). *C. stellifera* is unique in its red colouration and single star pattern placed at the apex of the shell. This species is more readily noticed by the total shell coverage of encrusting algae.

Cellana limpets are dominant grazers on rocky intertidal platforms throughout New Zealand and form part of the herbivorous guild of limpets that dominate rocky systems throughout the world (Lindberg and Hickman, 1986). The New Zealand limpet guild is composed of Fissurelliidae, Siphonariidae, Acmaeidae, Lottididae, Patellidae, Lepitidae and Nacellidae (Powell, 1979). Limpets are often the most abundant organisms throughout the intertidal zones, and impart the greatest effect on structuring the intertidal community by maintaining the distribution and abundance of the habitat-forming seaweeds (Creese and Underwood, 1982).

The biogeographic distribution of the New Zealand *Cellana* species varies across the genus (Fig.1.2) although all species may co-exist on some platforms (Powell, 1979). Three species (*C. ornata* and *C. radians* and *C. stellifera*) are distributed around the entire coastline of New Zealand's mainland, including Stewart Island (Fig. 1.2). *C. ornata* is mostly restricted to the mid – high intertidal zone whereas *C. radians* can also be found in the lower intertidal zone. *C. stellifera* is the only subtidal *Cellana* species, occurring throughout the subtidal reefs of New Zealand. This species is considered to be rarer than the other species, although an accurate measure of its abundance is difficult to obtain due to the subtidal nature of the species. *C. flava* and *C. denticulata* have a more restricted distribution (Fig. 1.2), occurring on the east coast only and are present mostly on outer reefs and headland areas. There is a very distinct northern cut-off in distribution around the Bay of Plenty, although smaller populations have been recorded on islands throughout the Bay of Plenty and the Three Kings Islands. The southern-most population of *C. flava* occurs around Motunau Island, north of Banks Peninsula, while *C. denticulata* is reported to occur as far south as Banks Peninsula. *C. strigilis redimiculum* is restricted to the southeast of the South Island, extending as far north as Timaru and south to Stewart Island. This species is found in the mid-low intertidal zone on reefs and boulders. Four other sub-species of the *C. strigilis* complex are found on the sub-Antarctic islands of New Zealand and the Chatham Islands.

Aside from the variation in biogeographic distribution these species are similar in many respects. All species coexist on rocky shore platforms and live for up to seven years with most lasting only 2-3 years (Dunmore and Schiel, 2000). All species graze on microspores of macroalgae (Creese, 1988). *Cellana* species are thought to have a short dispersive phase of around 3 – 11 days, reproducing by broadcast spawning after one year of development (Dunmore and Schiel, 2000). Based on the large yolky appearance of *Cellana* eggs (Jaeckle, 1995) and the larval longevity of the sister taxon *Patella* (Branch, 1981; Fretter and Graham, 1994) nacellids are thought to have restricted dispersal potential. A lecithotrophic period of 2 – 11 days follows the free spawning of gametes and a non-feeding trochophore stage of 1 – 2 days. Anderson (1962) showed that *C. tramoserica* larvae were spawned and settled within 2 days but an attempt by Balaparameswara Rao (1975) to spawn and raise larvae of *C. radiata* failed after 48 hours of development of the veliger stage

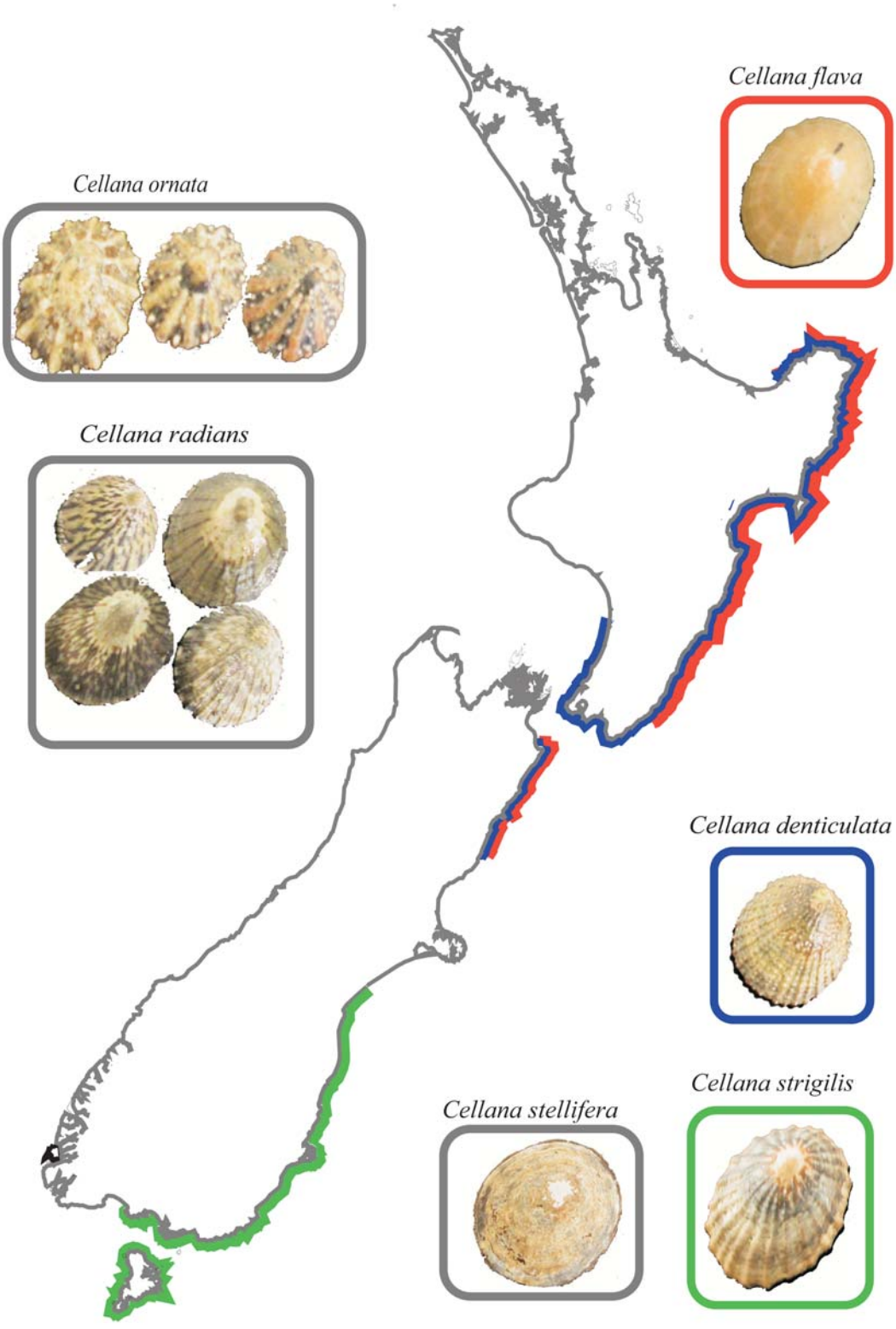

Figure 1.2. Biogeographic distribution of six New Zealand *Cellana* species. A reference picture for each species is given and the border colour corresponds to the distribution on the map of New Zealand.

Spawning time differs between the species. Gonad indices indicate that *C. ornata* spawns late in the Summer (Feb – March)(Dunmore and Schiel, 2000), while *C. flava* spawns during Spring and early Summer (Walters, 1994). *C. radians* and *C. denticulata* both have extended spawning seasons with a short resting stage (Creese and Ballantine, 1983; Walters, 1994). Gonad indices have been shown to vary between sites for *C. ornata*, suggesting a variation in the spawning time between populations, although a single spawn event was maintained at both sites (Dunmore and Schiel, 2000). Disparity in the time of spawning between populations has also been shown for the Australian *Cellana* species *C. tramoserica* (Parry, 1982).

Estimates of fecundity in separate studies suggest that *C. ornata* releases 1.5 - 2 times the number of eggs released by *C. radians* and up to three-fold more than *C. flava* and *C. denticulata* in one spawn episode (Dunmore and Schiel, 2000). The number of eggs released in one episode ranges from c. 20 x 10^3 (*C. flava* and *C. denticulata*) to 230 x 10^3 for *C. ornata*. However, it was noted that *C. radians* and *C. denticulata* had multiple spawnings per year (Dunmore and Schiel, 2000).

The biogeographic and demographic similarities across the New Zealand species of the *Cellana* genus make these limpets good target organisms for the investigation of phylogeographic hypotheses based on New Zealand biogeographic classifications and the well-characterised oceanographic and palaeogeographic features of New Zealand's coastal environment.

1.10 Thesis Outline

Chapter II is a phylogenetic study of the *Cellana* genus which includes published data from species throughout the global distribution of the genus. The resulting phylogeny is used to discriminate between two biogeographic hypotheses. Hypotheses tested:

- *Cellana* is a warm water taxon, originating in northern waters of the Indo-Pacific.
- ancestral *Cellana* spp. arose via allopatric speciation through separation from Gondwana, Australia and Antarctica.

Chapter III is a comparative investigation of the phylogeographic structure of three of the New Zealand *Cellana* species. *C. ornata*, *C. radians* and *C. flava* are used in this chapter as the biogeographic range of these species spans Cook Strait. Cook Strait is proposed as a dispersal barrier in this region. Tested hypothesis:

- A physical barrier to marine dispersal is influencing the genetic structure of limpet populations around Cook Strait.

Chapter IV again uses comparative phylogeography to test an hypothesis of dispersal. In this chapter the distribution of genetic variation and diversity of *C. ornata* and *C. radians* are compared. Tested hypothesis:

- The distribution of genetic variation for *Cellana* species is concordant with the major sea surface currents around New Zealand

Chapter V presents a general discussion in which intraspecific genetic studies on the coastal marine taxa of New Zealand are reviewed. Phylogeographic concordance across studies that have used mitochondrial DNA analyses to show phylogeographic discontinuities around the Cook Strait region is also investigated and biogeographic concordance is assessed.

Chapter II

Molecular Phylogenetics and Biogeography of New Zealand Nacellid Limpets

Nacellid limpets of the genus *Cellana* are the dominant grazers on rocky intertidal platforms around New Zealand. Some species have distributions that span the country while others are more restricted in their occurrence. Using partial sequence of the mitochondrial 12S and 16S ribosomal RNA genes, I examined phylogenetic relationships among *Cellana* species and used these data to contrast phylogeographic hypotheses about the origin of New Zealand species. Relationships and divergence time estimates were obtained using maximum parsimony and LogDet analyses which were then calibrated to limpet fossil records. Based on the phylogenetic relationships of 6 New Zealand and 13 Indo-West Pacific *Cellana* species I suggest that *Cellana ornata* may be characteristic of New Zealand's founding lineage, present at the time of separation of Gondwana.

2.1 Introduction

The origin and radiation of nacellid limpets is not well-resolved, but fossil records provide evidence of a historical range spanning throughout the Pacific, including North America (Lindberg and Hickman, 1986), north and south Australia, New Zealand, Java, Chile and east and west Antarctica (Powell, 1973). The modern biogeographic range of *Cellana* is mostly tropical, extending north to Japan, east to Juan Fernandez and Hawaii, south to South Africa, Madagascar, south east Australia, New Zealand and the sub-Antarctic Islands, where it co-exists on Campbell Island with the sister taxon *Nacella* (Powell, 1973). *Nacella* occurs within the sub-Antarctic and Megallenic region (Powell, 1973).

Molecular phylogenetic studies of patellogastropods (Koufopanou et al., 1999; Nakano and Ozawa, 2004) have confirmed the monophyletic grouping of the Nacellidae limpets (genera, *Nacella* and *Cellana*) relative to the sister taxon Patellidae. The Nacellidae classification was first proposed in 1975 by Golikov and Starobogatov (1975), but was not widely accepted until Lindberg and Hickman (1986) confirmed the familial split through shell microstructure analysis.

Eocene fossil deposits from Oregon, USA (Lindberg and Hickman, 1986) have been used for calibration of the minimum separation time between *Nacella* and *Cellana* (Koufopanou et al., 1999; Nakano and Ozawa, 2004). Powell (1973) suggested that the separation of *Patella* and *Cellana* was established before the Eocene. These proposed timings plus other tentative fossil assignments suggest that the Nacellidae are an ancient taxon.

New Zealand species of *Cellana* represent the southern most limits of the genus range and are geographically placed between three potential immigrant sources: the tropics, temperate Australia and the sub-Antarctics. Two species, *Cellana ornata* and *C. radians*, are distributed around the entire coastline of New Zealand. *C. flava* and *C. denticulata* are restricted to the north east coast while *C. strigilis redimiculum* is restricted to the south east. *C. stellifera* is the only sub-tidal species of this genus and is distributed around the entire coast of New Zealand. The *C. strigilis* complex includes six sub-species: *C. s. redimiculum* on the main land; *C. s. chathamensis* from the Chatham Islands and four *C. s.* spp on the sub-Antarctic Islands.

Molluscan diversity increased in New Zealand through the late Cretaceous and Cenozoic either from the original Gondwanan fauna or through immigration from elsewhere (Stevens, 1980; Beu and Maxwell, 1990; Cooper and Millener, 1993; Daugherty et al., 1993; Stilwell, 1997). Sea level changes are thought to have encouraged speciation and endemism within New Zealand (Fleming, 1979; Beu and Maxwell, 1990). Immigrants to New Zealand included Indo-Pacific marine taxa, although these taxa are almost exclusively species with teleplanic larvae capable of long distance dispersal and do not include limpet taxa (Fleming, 1979). Based on laboratory investigations of larval longevity of the sister taxon *Patella* (Branch, 1981), nacellids are thought to have restricted dispersal potential not conducive to oceanic transport. A lecithotrophic period of 7 – 11 days follows the free spawning of gametes and a non-feeding trochophore stage of 1 – 2 days. This short period of larval dispersal and the homing behaviour of the adults suggest low levels of continental interchange and high endemism for the genus.

Although there is no evidence to support a southern or northern origin of the nacellids, Powell (1973) suggested that *Cellana* is of warm water origin with relict populations in the higher latitudes. Nakano & Ozawa (2004) presented evidence to suggest that the circum-polar current had influenced the range expansion of the *Cellana* genus, while Koufopanou et al. (1999) tentatively supported a southern origin for *Cellana* and *Nacella* with subsequent northward and southward radiation of the genera, respectively.

In this chapter I have used molecular phylogenetic analyses to examine two contrasting hypotheses about the biogeographic origins of the New Zealand *Cellana* limpets:

1) *Cellana* is a warm water taxon, originating in northern waters of the Indo-Pacific

2) Ancestral *Cellana* spp. arose via allopatric speciation through separation from Gondwana, Australia and Antarctica.

The mitochondrial 12S and 16S ribosomal RNA (rRNA) genes were used to examine the two biogeographic hypotheses. The use of these genes allowed the incorporation of published sequences from previous studies of patellogastropods (Koufopanou et al., 1999; Nakano and Ozawa, 2004) and visualisation of ancient phylogenetic relationships, which fossil data suggest can be expected among limpet taxa.

2.2 Methods and Materials

2.2.1 Sample Collection

To incorporate intraspecific sequence variation, limpets were collected from sites from the north to the south of New Zealand and from the east and west coasts (Table A2.1), although only a sub-set of these samples were needed for analysis (Table 2.1). Wide ranging species (*Cellana ornata* and *C. radians*) were collected from around the country, while species with a more restricted distribution (*C. flava*, *C. denticulata*, *C. stellifera* and *C. strigilis*) were collected from multiple sites within their ranges. Collections were made during the period November 2002 to February 2003.

In general, five limpets were collected from each site (Table A2.1). However, larger samples were used for *C. s. strigilis* from Auckland and Campbell Island due to the restricted number of populations available for comparisons. Morphological variants of *C. radians* were used to cover the wide range of morphological variation in this species. Samples were preserved in 70% ethanol or frozen in liquid nitrogen, depending on the location and logistical constraints.

Sequences for *Cellana*, *Scutellastra* and *Nacella* of the Indo-West Pacific and Antarctica were obtained from GenBank (Table A2.2). The outgroup taxa, *Scutellastra flexuosa* was chosen from a recent Patellogastropod study (Koufopanou et al., 1999) because it exhibited the least difference in base composition with the nacellid limpets while maintaining sister taxa status.

Table 2.1. *Cellana* species and the collection site included in this study.

Species	Locality
Cellana denticulata (Martyn, 1784)	Kaikoura
Cellana flava (Hutton, 1873)	Cape Campbell
Cellana ornata (Dillwyn, 1817)	Kaikoura
Cellana radians (Gmelin, 1791)	Cape Campbell
Cellana stellifera (Gmelin, 1791)	Taupo Bay
Cellana strigilis redimiculum (Reeve, 1854)	Moeraki
Cellana strigilis chathamensis (Pilsbry, 1891)	Whangatete Inlet, Chatham Island
Cellana strigilis strigilis (Hombron and Jacquinot, 1841)	Campbell Island
	Auckland Island
Cellana radiata capensis (Gmelin, 1791)	Cape Vidal, South Africa
Cellana pricei Powell, 1973	O Le Pupu, Upolu, Westerm Samoa
Cellana solida (Blainville, 1825)	Oford, Tasmania, Australia
Cellana taitensis (Roding, 1798)	Tahiti, French Polynesia
Cellana tramoserica (Holten, 1802)	Mollimook, NSW, Australia
Cellana testudinaria (Linnaeus, 1758)	Vietnam
	Okinawa, Japan
Cellana radiata orientalis (Pilsbry, 1891)	Okinawa, Japan
Cellana grata (Gould, 1859)	Hong Kong
	Mie, Kaino, Japan
Cellana nigrolineata (Reeve, 1854)	Mie, Kaino, Japan
Cellana toreuma (Reeve, 1855)	Hong Kong
Nacella concinna (Strebel, 1908)	Signy Island, Antarctica
Scutellastra flexuosa (Quoy & Gaimard, 1834)	Savaii, Western Samoa

*Note: The collection locality for New Zealand includes only the single reference sequence used for analysis due to the lack of intraspecific variation. See table A2.1 for the complete list of New Zealand locations sampled and sequenced.

2.2.2 DNA Preparation

For each specimen a 3-5mm^2 section of muscle tissue was cut from the centre of the foot. The tissue was rinsed with distilled water and finely diced using flame-sterilized forceps and scissors. The diced tissue was digested and purified following a modified lithium chloride/chloroform protocol (Gemmell and Akiyama, 1996). DNA pellets were resuspended in 100µl TE8 (10mM Tris-HCL, pH8.0, 1mM EDTA) and stored at -20^{o}C.

Partial fragments of the 12S and 16S mitochondrial genes were obtained using 12Sma, 12Smb and 16LRN13398, 16SRHTB primer pairs (Koufopanou et al., 1999). PCR amplifications were done in a 25µl reaction volume, consisting of 1x buffer (50mM KCl, 10mM Tris-HCl, pH 8.0), 1.5mM $MgCl_2$, 200µm dNTP's, 0.54µm each primer, 0.5U Taq (Invitrogen), 12.9µl double-distilled, autoclaved water plus 2µl of template DNA. Thermal cycling parameters included an initial denaturation at 94^{o}C for 2 minutes, followed by 36 cycles at 94^{o}C for 20 sec, 47^{o}C (12S) and 51^{o}C (16S) for 20 sec, and 72^{o}C for 30 sec, before a final 7 minute extension at 72^{o}C. PCR products were purified with Millipore Montage PCR_{96} Multiscreen filter plates (Biolab, New Zealand).

Purified PCR products were sequenced in both directions with the 12Sma, 12Smb, 16LRN13398 and 16SRHTB primers, using a Big Dye V3.1 sequencing kit (Applied Biosystems, Foster City, CA) as per the manufacturer's instructions. Sequence products were purified using Sephadex-GS50 gel filtration (Amersham Bioscience, New Zealand). Capillary separation of samples was done by the Alan Wilson Centre Genome Service on an ABI 3730 DNA analyser for 12S sequencing and by University of Canterbury Sequencing Service on an ABI 3100 DNA analyser for 16S sequencing. Sequences were deposited in the NCBI GenBank (Table A2.2).

Multiple alignment of all sequences (Table A2.3; Table A2.4) was performed using default parameters in ClustalX (Thompson et al., 1997): *Cellana* and *Nacella* sequences were aligned and then a profile-to-profile alignment was done with the outgroup taxa; maintaining secondary structures and conserved motifs (Hickson et al., 1996; Lydeard et al., 2000).

2.2.3 Analysis

Phylogenetic analysis

Analysis of the 12S and 16S data sets was done separately and congruence between them was confirmed with a partition homogeneity test (Farris et al., 1995) for a combined analysis in PAUP*4.0b10 (Swofford, 1998). Nucleotides identified as stem sequence, based on the 12S and 16S secondary structures (Hickson et al., 1996; Lydeard et al., 2000) were initially down-weighted by 20% (Wheeler and Honeycutt, 1988; Dixon and Hillis, 1993). However, analyses run without weighting of stems did not change the results so weighting was eliminated from further analysis of these data. Maximum parsimony and LogDet distance (Steel et al., 2000) analyses were conducted on the combined data set with 10,000 bootstrap replicates using PAUP*4.0b10 (Swofford, 1998). Maximum Likelihood analysis was performed with 100 bootstrap replicates using PAUP*4.0b10 (Swofford, 1998). The GTR + I + G (I, 0.29; α, 0.37) model of evolution was used in maximum likelihood analysis as determined by Modeltest v. 3.06 (Posada and Crandall, 1998) following the Akaike Information Criteriton (AIC; (Sakamoto et al., 1986). Phylogenetic signal was confirmed with the g_1 statistic (Hillis and Huelsenbeck, 1992).

Tree Topology Tests

The Kishino-Hasegawa test of tree topology (Kishino and Hasegawa, 1989), using maximum likelihood scores was applied to six constrained trees (Fig. 2.1) and run using PAUP*4.0b10 (Swofford, 1998). Two trees tested the monophyly of New Zealand species (Fig. 2.1; T1, T2), while the remaining four trees were representative of the contrasting *a priori* hypotheses of biogeographic origin, assuming monophyly (Fig. 2.1; T3, T4) and polyphyly (Fig. 2.1; T5, T6) of the New Zealand species.

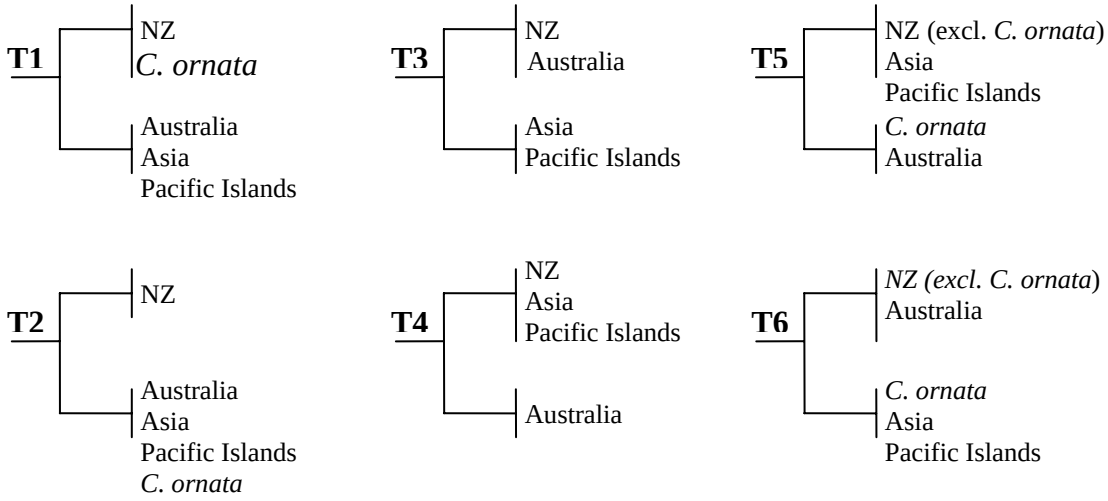

Figure 2.1. Constrained tree topologies for maximum likelihood comparisons using the Kishino-Hasegawa test. Trees 1 (T1) and T2 test for monophyly across the New Zealand species. T3 –T6 test *a priori* hypotheses of genetic association between New Zealand and the Pacific and Australia.

Molecular Clock assignment

The assignment of a molecular clock requires consistency among data to ensure that assumptions of constant evolution are not violated (Tajima, 1993). Tests of base composition and nucleotide change as well as evolutionary rate estimation were conducted on the New Zealand sequence data in MEGA version 2.1 (Kumar et al., 2001) for each gene separately. Tajima's Relative Rates test (Tajima, 1993) was performed using *S. flexuosa* as the outgroup taxon. Pairwise transition and transversion differences were calculated separately and plotted against pairwise LogDet distances to test for substitution saturation within the data. Pattern homogeneity (Disparity Index) with Monte-Carlo testing (Kumar and Gadagkar, 2001) was conducted in MEGA version 2.1 (Kumar et al., 2001) to confirm base composition consistency among taxa. A Neighbour-Joining tree with 10,000 bootstrap replicates based on pairwise LogDet

distance was transformed to a linearized tree to estimate divergence times between clades (Takezaki et al., 1995). The fossil record from Oregon (Lindberg and Hickman, 1986) was used to calibrate the rate of divergence between *Cellana* and *Nacella*, setting the divergence date at 38 million years ago (mya). Two fossils from the Late Eocene (38 mya) in New Zealand show similarities to present day *C. strigilis* and *C. denticulata* (Powell, 1973; Beu and Maxwell, 1990). The shell microstructure of these fossils has not been examined and so allocation to the genus *Cellana* is tentative. However, alternative rates were obtained by initially setting 38 mya as the minimum divergence time of the New Zealand clade and a second rate was obtained by setting the minimum appearance of *C. ornata* as 38 mya.

2.3 Results

360 and 490 base pairs of mitochondrial 12S and 16S rRNA sequences respectively were resolved for each of the New Zealand species (Table 2.1). There was no intraspecific variation in either gene. When combined with sequences from GenBank the total aligned sequence lengths were 260 and 419 bp, respectively (Table A2.1).

Phylogenetic analysis

Partition homogeneity tests showed congruence ($P > 0.1$) between the 12S and 16S data (Fig. 2.2), so phylogenetic analysis was done on the combined data set. Analysis for the combined data was based on 675 bp, comprising 284 variable sites, 171 of which were parsimony informative. The g_1 statistic showed that a significant phylogenetic signal was obtained ($P < 0.01$).

Maximum Parsimony and Maximum Likelihood analysis revealed a polyphyletic topology for the New Zealand species (Fig. 2.3 and Fig. 2.4). A monophyletic clade composed solely of New Zealand limpets, was supported in 99% of bootstrap replicates. However, this clade did not include *C. ornata* which stands unresolved with weak bootstrap support (35%) placing it in a basal position within this genus.

Although the New Zealand clade was clustered with Australian species (Fig. 2.3) the low resolution of the data did not confirm whether these species are

phylogenetically closer than the Asian or Pacific Island species. Maximum Likelihood and LogDet analyses show alternative clustering is also possible (Fig. 2.4 and Fig. 2.6). This was further supported by the Kishino-Hasagawa tests which were non-significant ($P > 0.5$) for all six tree topologies.

Four other clades were well supported by both maximum likelihood and maximum parsimony analyses (Fig. 2.3 and Fig. 2.4). An Asian Clade (B) was well supported at 95% and 89%, and was composed of two species restricted to Japan and Hong Kong. Clade C included *C. radiata capensis* (SA) and *C. radiata orientalis* (Japan) occurring together 95% and 85% of the time. Taxonomic classification suggests that *C. r. capensis* and *C. r. orientalis* are sub-species of *C. radiata* (Powell, 1973) and while the data are insufficient to explore this, they lend support to a close taxonomic relationship between these species. A Pacific Island lineage, D, included Tahiti and Western Samoa and has a bootstrap support of 91% and 97%.

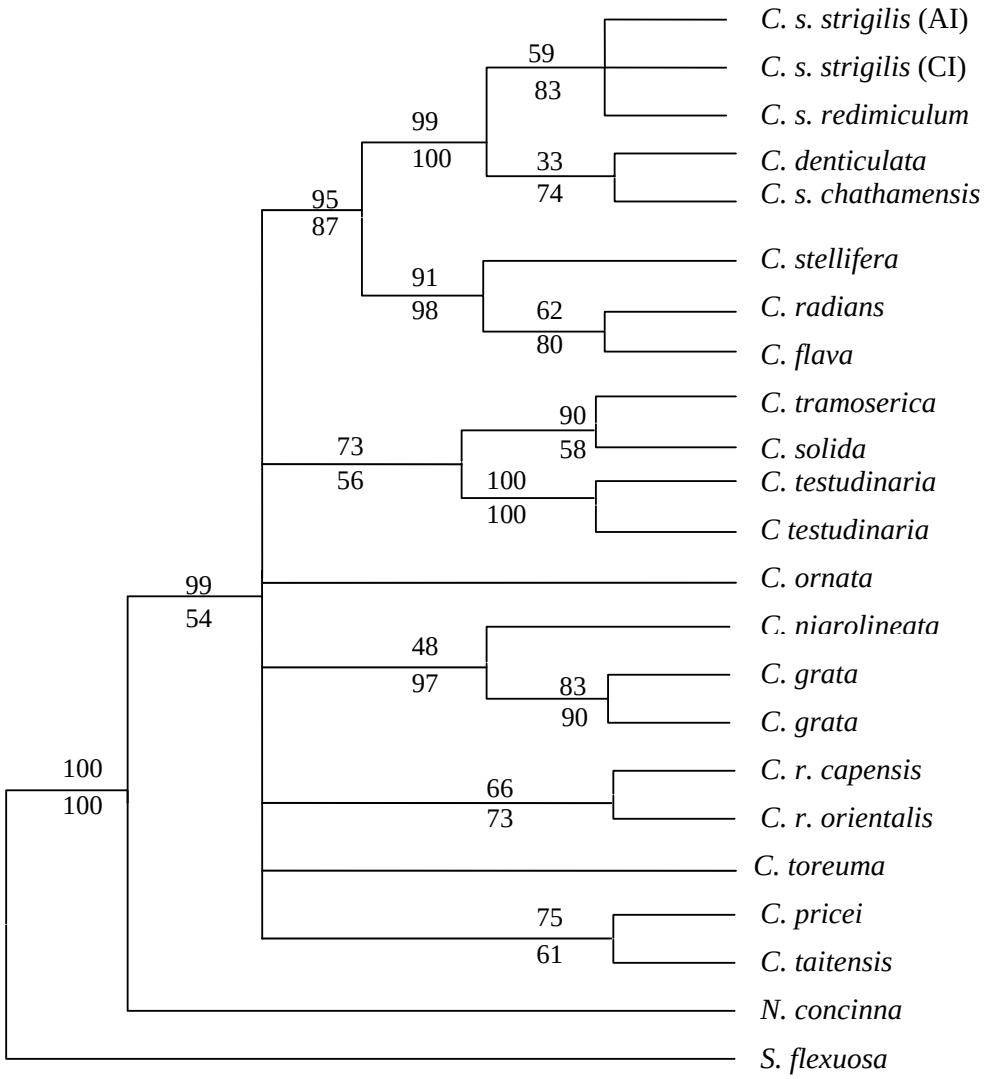

Figure 2.2. Maximum parsimony tree of mitochondrial 12S and 16S rRNA sequences. Bootstrap support values are shown above (16S) and below (12S) branches. The partition homogeneity test shows no significant difference between these data sets (P > 0.1). Abbreviations: C, *Cellana*; C. s., *Cellana strigilis*; C. r., *Cellana radiata*; N, *Nacella*; S, *Scutellastra*; AI, Auckland Islands; CI, Campbell Islands.

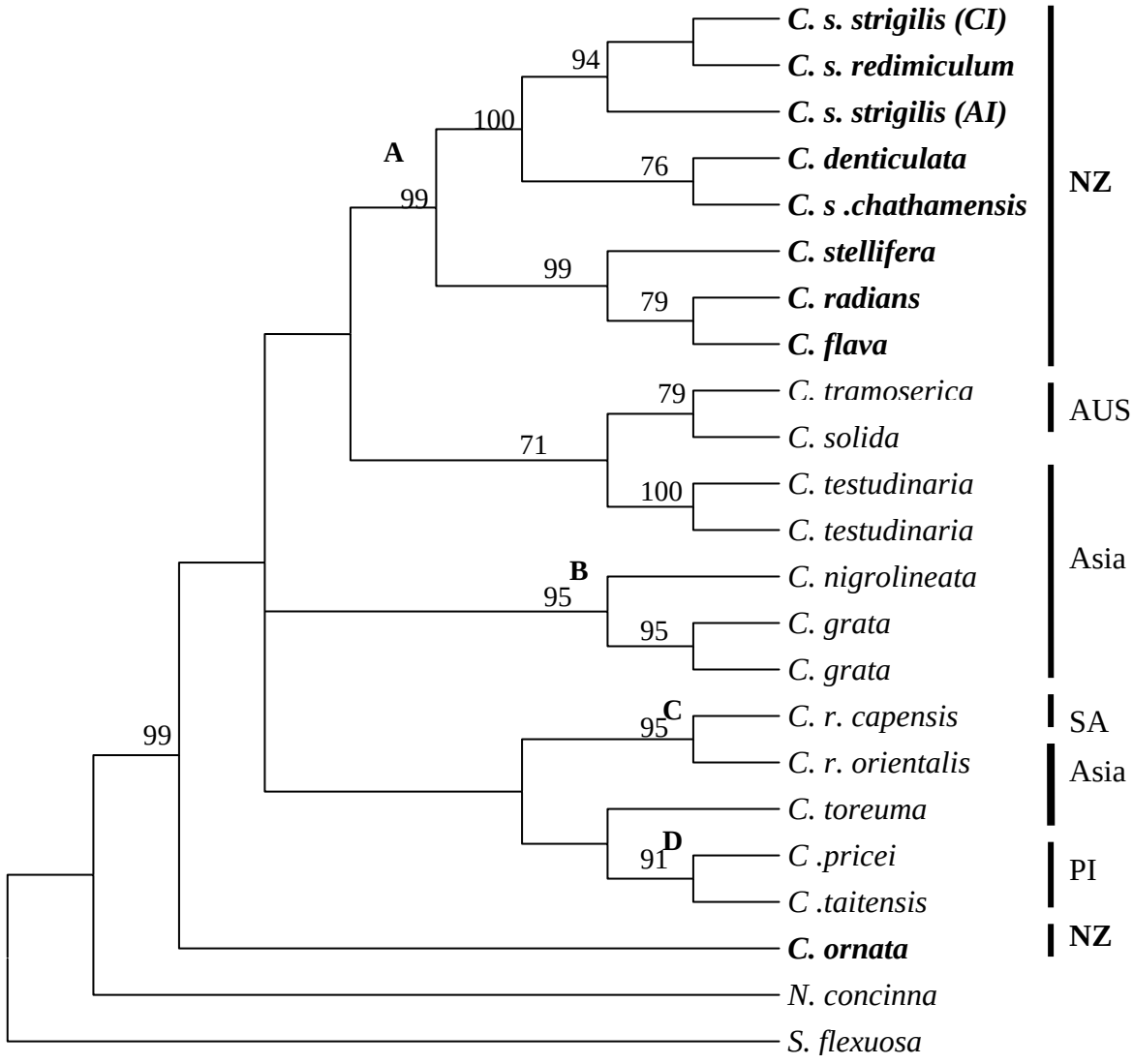

Figure 2.3. Maximum parsimony tree of combined 12S and 16S rRNA data. Bootstrap support values for 10,000 replicates are shown above the branches: values of less than 50% are not shown. Abbreviations: C, *Cellana*; C. s., *Cellana strigilis*; C. r., *Cellana radiata*; N, *Nacella*; S, *Scutellastra*; AI, Auckland Islands; CI, Campbell Islands; NZ, New Zealand; Aus, Australia; SA, South Africa; PI, Pacific Islands; Asia includes Japan, Hong Kong and Vietnam. New Zealand taxa are shown in bold. Clades A – D are identified.

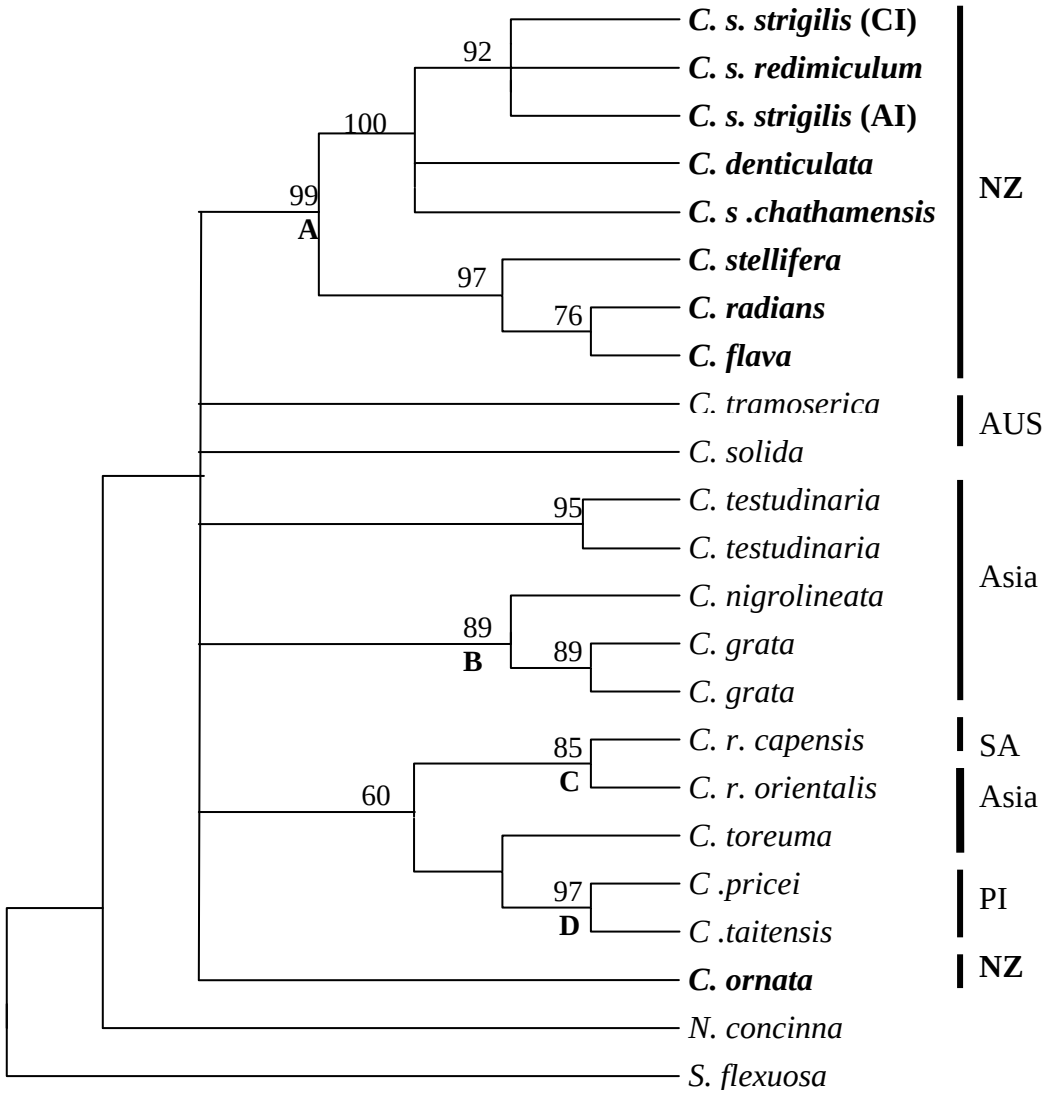

Figure 2.4. Maximum likelihood tree of combined 12S and 16S rRNA data. Bootstrap support values for 100 replicates are shown above the branches: values of less than 50% are not shown. Abbreviations: C, *Cellana*; C. s., *Cellana strigilis*; C. r., *Cellana radiata*; N, *Nacella*; S, *Scutellastra*; AI, Auckland Islands; CI, Campbell Islands; NZ, New Zealand; Aus, Australia; SA, South Africa; PI, Pacific Islands; Asia includes Japan, Hong Kong and Vietnam. New Zealand taxa are shown in bold. Clades A – D are identified.

Divergence time estimates

Pattern homogeneity tests for several taxa pairs showed significant disparity in nucleotide composition for both genes (Table A2.4 and Table A2.5), mostly between ingroup taxa and *S. flexuosa*, but also between *C. r. orientalis* and *C. pricei*, *C. taitensis* and *C. flava* and between *C. flava* and *C. radians*. LogDet distances were calculated, as this method is robust to compositional heterogeneity (Steel et al., 2000). Substitution saturation was not evident (Fig. 2.5) and the transition/transversion ratio was 1.6 (12S) and 1.7 (16S). Tajima's Relative Rates test (Tajima, 1993) showed no significant difference between ingroup taxa for either gene.

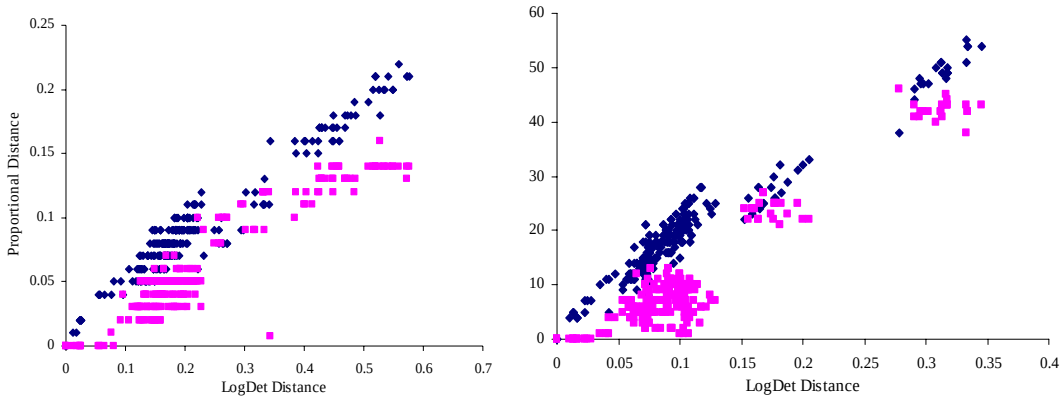

Fig 2.5. Transition and Transversion plots showing linearity between LogDet distance and pairwise substitutions for 12S (A) and 16S (B) rRNA sequences. Diamonds – Transitions; Squares – Transversions.

The three estimated divergence rates (Table 2.2) show that placing datum at the *Nacella*/*Cellana* split increases the rate of divergence and is less compatible with the published rates (Koufopanou et al., 1999) shown in table 2.2, than the rates obtained from the New Zealand fossil data. Using the divergence of the New Zealand clade for the placement of the 38 million year calibration point (Fig. 2.6) gives the most compatible rates (Table 2.2).

Table 2.2 Contrasting divergence rates expressed as nucleotide changes per site per million years.

Point of Calibration	12S	16S	Combined
Cellana/ *Nacella* – 38 mya	0.0031	0.0023	0.0026
NZ clade divergence – 38 mya	0.0016	0.00088	0.0012
C. ornata appearance – 38 mya	0.0021	0.0014	0.0015
Patellids Koufopanou et al. (1999)	0.0016	0.0014	
Littorina Koufopanou et al. (1999)	0.0011	0.00084	

Note: "Point of Calibration" represents the node used for the 38 million year calibration point. The rate for Patellids was estimated using the *Nacella*/ *Cellana* split for the 38 million year calibration point. Littorina is added for comparison.

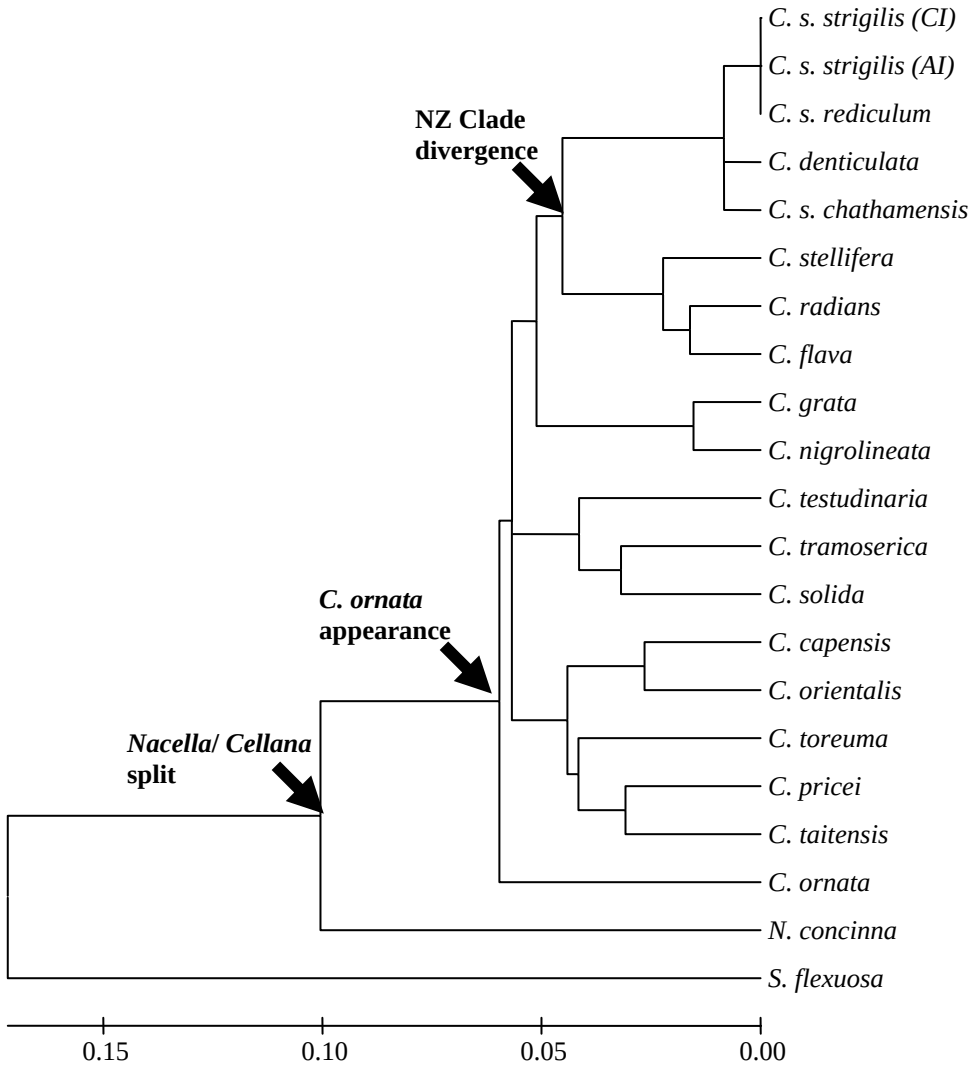

Figure 2.6. Linearized Neighbour-Joining tree based on LogDet distance analysis of the combined 12S and 16S sequence data. Arrows highlight the nodes used for the calibration of divergence rates.

2.4 Discussion

The aim of this study was to test two contrasting hypotheses of nacellid entry into New Zealand: entry by dispersal from the Indo-West Pacific versus allopatric fragmentation from Gondwana stock. I hypothesised that the addition of New Zealand species, the most southern limpets of the genus should help to resolve these opposing hypotheses.

The unresolved nature of the New Zealand species was unexpected. All the species, aside from the subtidal *C. stellifera* occur in the same habitat with up to five species co-occurring on the same platform. The polyphyletic topology observed suggests that *C. ornata* may basal to New Zealand and possibly the entire genus or that a second colonisation event may have bought a second ancestor into New Zealand, from which recent diversification has occurred. Alternatively, speciation occurred within New Zealand millions of years after the entry of the *C. ornata* like ancestor.

Comprehensive phylogenetic studies of the Littorininae (Reid et al., 1996; Williams et al., 2003) show many consistencies with this study. Here too molecular data were unable to resolve conclusively among competing biogeographic hypotheses. Williams et al. (2003) suggests that the lack of resolution for the main Littorininae genera may be due to rapid differentiation. Like *Cellana,* fossils pre-dating the Eocene have not been found for littorinines, but molecular data have the group as late Cretaceous age. However, unlike *Cellana* where the radiations occured within regions, the phylogenetic reconstruction for *Littorina* showed a mixture of tropical and temperate clades, which may be a result of diversification into new habitats.

Koufopanou et al. (1999) have shown, through molecular analysis, that *Patella s. s.* occurred much earlier than the first confirmed fossil of the Pliocene. The rates obtained in this study using the New Zealand fossil data are more similar to the evolutionary rates observed for the patellids and *Littorina* (Reid et al., 1996; Koufopanou et al., 1999) than assuming that the Oregon fossils represent the first appearance of the *Cellana* genus. The altered dates give a *Nacella*/*Cellana* split time of approximately 107 million years, pushing the split of this family back into the Cretaceous. This date matches tentative fossil records of Northern Australia (Powell, 1973) and coincides with a time of continental movement splitting Antarctica, Africa and India (Hay et al., 1999).

It is tempting to pick a date and speculate about the palaeoclimatic effects on speciation of the genus. However, the issue of fossil assignment and synapomorphic characters have yet to be fully resolved (Koufopanou et al., 1999). In addition, the tree topology shows no significant genetic linkage with Australia or Asia. It is possible that a more complete nacellid data set, including all Pacific Island species and the full *C. radiata* complex, plus extra *Nacella* samples might further resolve phylogenetic relationships. Although, Williams et al. (2003) used a comprehensive data set and were still unable to fully resolve the phylogenetic relationships of *Littorina*. Micro-structure analysis of limpet fossils would also allow for more accurate dating of speciation events by confirming the date and genus of the fossils in Australia and New Zealand that are currently assigned to the *Cellana* genus.

Chapter III

The Phylogeographic structure of *Cellana* limpets of New Zealand: Is Cook Strait a Barrier to Dispersal?

Distinct biogeographic boundaries for marine taxa in New Zealand have been recognised since the early 1900's when biogeographic provinces were identified for a diverse array of taxa including molluscs, echinoderms, seaweeds and fish. Species disjunctions in these taxa are evident at both East Cape and Cook Strait. Here I use mitochondrial cytochrome *b* DNA sequences of *Cellana ornata*, *C. radians* and *C. flava* to assess the phylogeographic pattern of populations spanning Cook Strait and look for concordance of contemporary and historical phylogeographic processes. I sequenced 708 samples from 32 populations spanning the biogeographic ranges of the three species. Phylogeographic analyses of these sequences showed a strong genetic discontinuity occurring at Cook Strait, which is most evident in *C.ornata*. *C. radians* appears to have experienced a demographic bottleneck with a subsequent range expansion. Further analysis suggests that allopatric fragmentation of populations around Cook Strait is driving the phylogeographic structure in this region. In addition, French Pass samples from the South Island suggest that contemporary gene flow is occurring within Cook Strait.

3.1 Introduction

Dispersal barriers in the marine environment are the focus of much interest and speculation. The East Pacific Barrier has been a focal point since Darwin (1860) claimed it to be an "impassable barrier" for migration. It took 128 years of deliberation before research focused on obtaining evidence of larval transport within the region (Scheltema, 1988). Scheltema (1988) re-classified the impassable barrier as more a "filter" to larvae of short dispersal potential, allowing only long distance dispersal to occur. Large ocean basins are an obvious place for a potential filter for marine dispersal and research has focused on the genetic discontinuity and population structuring between ocean basins (Palumbi and Kessing, 1991; Williams and Benzie, 1998; Perez et al., 1999; Shaw et al., 2004), but what about smaller water bodies such as straits, channels, inlets and bays? How large does a body of water have to be before it acts as a "filter" to dispersal? Or are complexity and historical stability more important factors than size?

Much less work has been done to determine whether smaller bodies of water can present a barrier to marine dispersal, although there are some good examples. For instance, Barber et al. (2000) found that the stomatopod *Haptosquilla pulchella*, with a potential for long distance dispersal exhibited genetic discontinuity between Ocean basins at the Makassar Strait, Indonesia, even though this area has the potential to facilitate long distance dispersal. In contrast, Kirkendal and Meyer (2004) found that the Makassar Strait did not prevent connectivity among populations of the limpet *Patelloida profunda*. Unlike stomatopods, limpets have a short larval life that does not usually lead to long distance dispersal. In both of these studies historical association with sea level change and availability of suitable habitat throughout the Pleistocene explained the observed phylogeographic structure.

The association of the English Channel with the phylogeographic structuring of taxa has recently been observed in the polychaete *Pectinaria koreni*, (Jolly et al., 2005) and the red seaweed *Palmaria palmata* (Provan et al., 2005). The authors of both of these studies report the influence of temporary refugia during the last glacial maximum, 23 - 19 thousand years ago (kya), when the English Channel was no more

than a narrow trench (Hurd Deep), after which range expansions and re-colonisation took place.

Phylogeographic concordance across a diverse array of taxa spanning the Straits of Florida has identified this as an area of phylogeographic discontinuity. The Straits separate the Gulf of Mexico from the Atlantic Ocean. The taxa studied ranged from horse-shoe crabs and oysters to sea bass (Avise, 2004, and references there in). Similarly, historical isolation between populations of the Atlantic Ocean and the Mediterranean Sea through the Straits of Gibraltar has been observed in various taxa, including coastal vascular plants (Clausing et al., 2000)

Putative dispersal barriers have been identified around the coastal shores of New Zealand and across Cook Strait (Apte and Gardner, 2002; Star et al., 2003; Waters and Roy, 2004). Cook Strait has been a transient feature of the New Zealand land mass for millions of years and has only recently (10 – 5 kya) filled to the present depth and width following periods of marine transgression through the Pleistocene glaciation periods (Stevens et al., 1995). The historical association of Cook Strait with the phylogeographic structure of taxa has been studied in the terrestrial environment {Trewick, 2000 #354;Trewick, 2000 #355;Trewick, 2001 #349}. In contrast, genetic population studies on marine taxa in New Zealand tend to remain focused on the influence of tidal flow, upwelling and directional currents as putative dispersal barriers (Apte and Gardner, 2002; Waters and Roy, 2004).

Stevens and Hogg (2004) report one of the few studies to explore vicariance as a process acting on marine structure in New Zealand. The authors suggest that populations of the coastal amphipod, *Paracorophium excavatum*, were once isolated in "lakes" in the Cook Strait region prior to the separation of the North and South Island and the formation of the Strait. However, it is not unreasonable to suggest, as in other New Zealand studies (Apte and Gardner, 2002; Waters and Roy, 2004) that hydrographic factors exert a major influence on the connectivity between the North and South Islands.

Upwelling events in particular occur when strong, persistent winds force along-shore flow to drive the deeper water to the surface around the coast and moves the surface layer of water offshore (Heath, 1972; Shirtcliffe et al., 1990), potentially transporting larvae away from suitable habitat (Schiel, 2004). Intermittent upwelling

is also associated with periods of relaxation which can in turn transport larvae onshore to coastal habitats (Schiel, 2004). Ocean flow barriers have the potential to constrain dispersal in a directional manner (Gaylord and Gaines, 2000). A well-known example of this is Point Conception on the Californian coast of the United States, where coastal flow and wind-induced upwelling is thought to function as a one-way barrier prohibiting northward transport around the point (Gaylord and Gaines, 2000). The Almeria-Oran Oceanographic Front (AOF) is a system of eddies and gyres at the boundary between Atlantic and Mediterranean waters. This front is another example of an ocean flow barrier causing discontinuities in marine invertebrate distribution and population connectivity (Rios et al., 2002).

Cook Strait exhibits a very complex system of converging water masses resulting in a sharp temperature interface with steep vertical gradients and irregular eddy formation (Heath, 1982; Vincent et al., 1991). Adjacent waters of the east and west coast are also complex in nature with anomalous wind-driven activity such as semi-permanent eddies and upwelling events dominating the region (Heath, 1972, 1982; Vincent et al., 1991; Chiswell and Roemmich, 1998; Uddstrom and Oien, 1999; Chiswell and Schiel, 2001; Murphy et al., 2001). However, it is unclear whether the coastline between Cape Campbell and Kaikoura is dominated by wind-driven upwelling events, "wisps" of sub-Antarctic water (Shaw and Vennell, 2000), offshoots of warm-core eddies from the north (Uddstrom and Oien, 1999; Shaw and Vennell, 2000), or the northward flow of the Southland current providing mixed coastal and sub-Antarctic water from the south (Heath, 1972, 1982; Uddstrom and Oien, 1999; Murphy et al., 2001)

Intraspecific phylogeography of New Zealand rocky shore invertebrates and commercial fish stocks often exhibits a greater degree of genetic structure than expected from species with high dispersal potential (Smith et al., 1989; Apte and Gardner, 2002; Sponer and Roy, 2002; Waters and Roy, 2004). In some of these studies Cook Strait (Fig. 3.1) appears to act as a barrier to dispersal between North and South Island populations (Apte and Gardner, 2002; Waters and Roy, 2004), with moderate phylogenetic discontinuity observed around this region, although the exact location of the discontinuity varies as a consequence of varying sampling locations.

This means the location of the phylogeographic discontinuity (or break) is yet to be determined.

Biogeographic boundaries and regional provinces have been described for molluscs, echinoderms, seaweeds and fish since the early 1900s and provided the first evidence of a biogeographic discontinuity between the North and South Islands of mainland New Zealand (Chapter V). Sponer and Roy (2002) recently re-visited the biogeographic provinces of the echinoderms when they examined the genetic population structure of *Amphipholis squamata*. Sponer and Roy (2002) used sequence and restriction-fragment length-polymorphism (RFLP) data of nuclear and mitochondrial DNA from 16 populations of *A. squamata* throughout New Zealand. The authors reported a genetic break occurring between the populations of Kaikoura and Banks Peninsula (Fig. 3.1) and they group South Island populations from Nelson (Fig. 3.1) and Kaikoura with North Island populations.

Mladenov et al. (1997) examined the genetic differentiation of another echinoderm, *Evechinus chloroticus*, from six populations around New Zealand using polymorphic allozyme loci. They found low genetic differentiation throughout the range of the species including between the North and South Island, suggesting dispersal across Cook Strait.

The most comprehensive studies to date involve the green-lipped mussel *Perna canaliculus*. Smith (1988) first used allozyme variation at 10 polymorphic loci on juvenile *P. canaliculus* from six locations around New Zealand. This study reported a significant difference between the two northern-most populations and the other four populations. This led Smith (1988) to infer that *P. canaliculus* exhibited thermal, genetic-physiological adaptation and isolation by oceanic currents. More recently, Apte and Gardner (2002) and Star et. al (2003) used various genetic techniques to re-address the genetic distribution of *P. canaliculus*. Apte and Gardner (2002) sampled the NADH IV mtDNA region from 22 sites throughout New Zealand using single-strand conformation polymorphism (SSCP) analysis. Like Smith (1988) they found genetic differentiation between a northern and southern group. The genetic discontinuity occurred immediately south of Cook Strait, leading the authors to infer that upwelling, strong tidal flow and turbulent mixing of water surrounding Cook Strait (Fig. 3.1) creates a physical barrier to dispersal. Further support for the

north-south discontinuity came from a study of 19 populations of *P. canaliculus*, using randomly amplified polymorphic DNA (RAPD) analysis (Star et al., 2003) where again a genetic discontinuity between northern and southern populations immediately south of Cook Strait was observed.

The most recent study to report the existence of a dispersal barrier in the Cook Strait region was that of Waters and Roy (2004) on the sea-star *Patiriella regularis*. In this study, 19 populations were sampled around New Zealand and the mitochondrial control region was analysed for population differentiation. As for the green-lipped mussels, *Patiriella regularis* exhibited a genetic break south of Cook Strait, leading the authors to reach the same conclusion that upwelling south of Cook Strait (Fig 3.1) presents a barrier to dispersal.

In this chapter I examine the population structure of three species of limpets of the *Cellana* genus (*C. ornata*, *C. radians* and *C. flava*) and use these data to examine phylogeographic processes. These three species were chosen because of their biogeographic distributions around New Zealand (Powell, 1979). All three species span Cook Strait. *C. ornata* and *C. radians* occur on all rocky intertidal platforms of New Zealand and are found in high abundance at all locations. *C. ornata* is restricted mostly to the mid – high intertidal zone, whereas *C. radians* can also be found in the low intertidal zone. *C. flava* has a more restricted distribution, occurring on the east coast only, and is mostly restricted to outer reefs and headland areas that are not readily accessible to sample. The southern-most population of *C. flava* occurs around Motunau Island, north of Banks Peninsula.

Despite some variation in biogeographic distribution these three species are similar in many respects. They all have short larval lives of around 3 – 11 days and reproduce by broadcast spawning. All species coexist on the rocky shore.

Spawning times differ between the species. *C. ornata* spawns in the late Summer (Feb – March) (Dunmore and Schiel, 2000), while *C. flava* spawns during the Spring and early Summer (Walters, 1994). *C. radians* has multiple spawning events throughout the year (Creese and Ballantine, 1983).

The aim of this chapter is to use these three species to test the phylogeographic hypothesis that a physical barrier to marine dispersal is influencing the genetic structure of populations around Cook Strait.

3.2 Materials and Methods

3.2.1 Sample Collection

For phylogeographic comparisons, only species with ranges spanning Cook Strait were examined. Four species of *Cellana* fit this criterion: *C. ornata* and *C. radians* are widely distributed around New Zealand (*C. ornata* and *C. radians*) while *C. flava* and *C. denticulata* are restricted to the east coast of New Zealand, north and south of Cook Strait. These four species were collected from intertidal rocky reefs throughout their range (Fig. 3.1, Table 3.1,). A sample size of 10 – 20 individuals per species from each location was collected. This sample size, within populations, was a trade-off for wide population coverage of multiple species. *C. radians* sample sizes were often greater than other species (Table 3.1) because of the extensive morphological variation in this species (Chapter I). Collections were made from November 2002 to February 2004. Samples were preserved in 70% ethanol. *C. denticulata* was collected but was not successfully sequenced, and so was removed from this study.

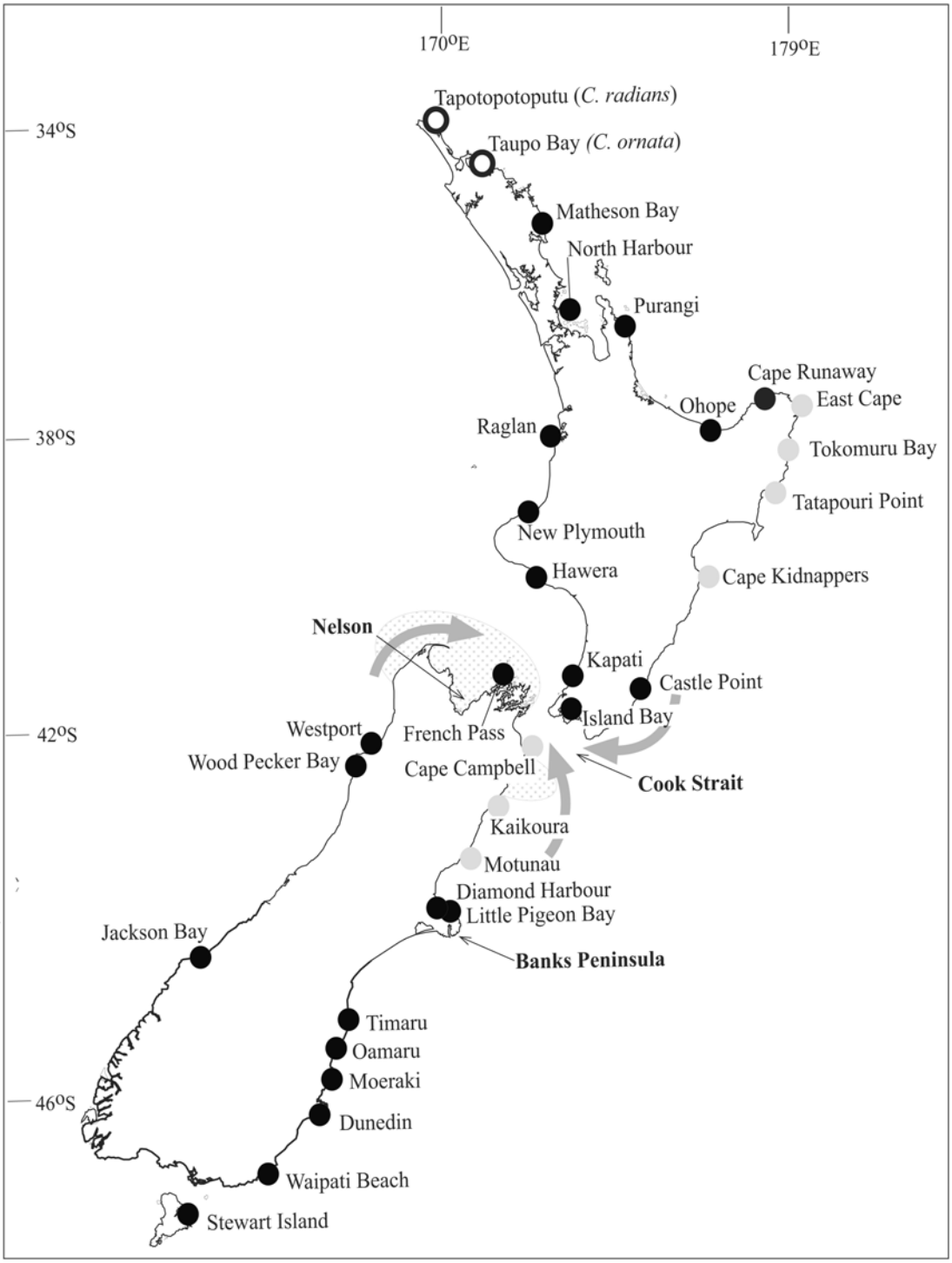

Figure 3.1. Study sites around New Zealand at which *C. ornata*, *C. radians* and *C. flava* samples were collected (black circles). Grey circles: locations where all three species were collected. *C. flava* was absent from all other locations; empty circles: one species only collected. Locations mentioned in the text and hydrographic features of Cook Strait are shown. Grey stippling indicates the upwelling regions around Cook Strait.

Table 3.1. Population location and sample size for *Cellana* species studied.

Population	Code	Sample Size		
		C. ornata	*C. radians*	*C. flava*
Cape Campbell	CC	10	10	11
Dunedin	D	11	9	-
Diamond Harbour	DH	20	13	-
French Pass	FP	9	14	-
Jackson Head	JH	0	6	-
Kaikoura	K	10	4	9
Little Pigeon Bay	LPB	10	13	-
Moeraki	M	10	10	-
Motunau	Mot	10	9	8
Oamaru	Om	6	8	-
Stewart Island	SI	15	24	-
Timaru	Tim	9	11	-
Waipati Beach	WP	5	4	-
West Port	We	10	10	-
WoodPecker Bay	WPB	10	11	-
Cape Kidnappers	CK	11	14	10
Castle Point	CP	10	20	-
East Cape	EC	10	11	20
Hawera	Haw	10	5	-
Island Bay	IB	9	10	-
Kopongatahi Point	CR	10	10	8
Koutunui Point	Tok	10	10	10
Matheson Bay	MB	5	10	-
New Plymouth	NP	10	10	-
North Harbour	NH	10	10	-
Ohope	Oh	10	10	-
Pukerua Bay	Kap	10	7	-
Raglan	Rag	10	10	-
Taupo Bay	TB	10	0	-
Topotuputu	TTP	10	9	-
Tatapouri	Tat	10	12	9
Whitianga Harbour	P	10	7	-
Totals	**32**	**302**	**321**	**85**

Note: "Dash" indicates the absence of the species at the specified location.

3.2.2 DNA Preparation

For each specimen, a 3-5mm^2 section of muscle tissue was cut from the centre of the foot. The tissue was rinsed with distilled water and finely diced using flame-sterilized forceps and scissors. Diced tissue was then digested and purified following a modified lithium chloride/chloroform protocol (Gemmell and Akiyama, 1996): an additional LiCl step was added between the chloroform and ethanol steps (see also Appendix A1.1). DNA pellets were resuspended in 100µl TE8 (10mM Tris-HCl, pH8.0, 1mM EDTA) and stored at -20^{o}C.

Partial sequence of the mitochondrial cytochrome *b* gene was obtained using primers modified from those of Kocher et al (1989; Appendix A1.2). PCR amplifications were done in a 25µl reaction volume, consisting of 1x buffer (50mM KCl, 10mM Tris-HCl, pH 8.0), 1.5mM $MgCl_2$, 200µm dNTP's, 0.54µm each primer, 0.5U Taq (BioLine), 12.9µl double-distilled, autoclaved water plus 2µl of template DNA. Thermal cycling parameters included an initial denaturation at 94°C for 2 minutes, followed by 36 cycles at 94°C for 20 sec, 47°C for 20 sec and 72°C for 30 sec, before a final 7 minute extension at 72°C (Appendix A1.2). Amplification success and DNA quantification were determined by agarose gel electrophoresis on a 2% agarose gel with 0.5x TBE buffer (4.5mM Tris, 4.5mM boric acid, 1mM EDTA, pH 8.0), against a size marker (λ/EcoR1/Pst1) of known concentration. PCR products were purified with Millipore Montage PCR_{96} Multiscreen filter plates (Biolab, New Zealand).

PCR products were sequenced in both directions with species specific forward and reverse cytochrome *b* primers (Appendix A1.2), using a Big Dye v. 3.1 sequencing kit (Applied Biosystems, Foster City, CA) as per the manufacturer's instructions. Sequence products were purified using Sephadex-GS50 gel filtration (Amersham Bioscience, New Zealand). Capillary separation of samples was done by the Allan Wilson Centre Genome Service on an ABI 3730 DNA analyser and by University of Canterbury Sequencing Service on an ABI 3100 DNA analyser. Haplotype sequences were deposited in the NCBI GenBank database (Appendix Table A3.2.14.).

3.2.3 Sequence Analysis

Haplotypes were identified manually for *C. ornata* (Appendix Table A3.2.1), *C. radians* (Appendix Table A3.2.5) and *C. flava* (Appendix Table A3.2.10) using Bioedit v. 5.0.6 (Hall, 1997). Statistics were calculated and tests of selection were run using the program Arlequin v. 2.0 (Schneider et al., 2000). Tajima's (1989) test (D) examines the relationship between the number of segregating sites and the average number of nucleotide differences through pairwise comparisons. This test detects the presence of deleterious mutations or balancing selection (Tajima, 1989; Nei and Kumar, 2000). Fu's (1997) statistic (Fs) was developed to detect evolutionary forces

described by population genetic models of hitchhiking, growth and background selection and is based on the probability of having *k* alleles in a sample of *n* sequence, given the value of θ (Fu, 1997).

The average genetic diversity for each species was estimated using Nei's (1987) gene diversity (*h*) and nucleotide diversity (π) measures in Arlequin v. 2.0 (Schneider et al., 2000). These two measures of genetic diversity are simple measures of heterozygosity deemed appropriate for single locus sequences of low diversity (Nei and Kumar, 2000).

Statistical parsimony networks were constructed in TCS 1.18 (Clement et al., 2000). For each species, Modeltest v. 3.06 (Posada and Crandall, 1998) was used to obtain the evolutionary model best fitting the data. The Akaike Information Criterion (AIC; (Sakamoto et al., 1986) was followed. AIC has been shown to outperform log likelihood testing as it allows for model selection uncertainty and model averaging and does not require nesting of the models (Nei and Kumar, 2000; Posada and Buckley, 2004). The genetic distance between haplotypes was calculated in PAUP*4.0b10 (Swofford, 1998) using the maximum likelihood model suggested by Modeltest v. 3.06 (Posada and Crandall, 1998): the transversional model (TVM), assuming unequal variance was used for *C. ornata*. Tamura and Nei's (1987) model of nucleotide substitution (TrN) was used for *C. radians*. An evolutionary model could not be fitted to the *C. flava* data as it has no parsimony informative sites and only two nucleotide substitutions between the most divergent haplotypes, so the Jukes and Cantor model of nucleotide substitution (Jukes and Cantor, 1969) was used as this is the simplest model of nucleotide substitution.

3.2.4 Phylogeographic Structure

Genetic differentiation between populations was determined using Nei's (1987) uncorrected measure of nucleotide differentiation (d_{XY}). This measure of differentiation represents the average number of nucleotide substitutions per site between haplotypes from two populations (Nei and Kumar, 2000). These calculations were performed in Arlequin v. 2.0 (Schneider et al., 2000) and visualised with non-metric multidimensional scaling (MDS) using the program Statistica v. 6.

The hierarchical distribution of genetic variation among populations was tested using an Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) in Arlequin v. 2.0 (Schneider et al., 2000) based on the number of pairwise nucleotide differences (Schneider et al., 2000). This simple distance measure was used due to the close genetic relationship of the sequences (Nei and Kumar, 2000). AMOVA is a method of partitioning the data into subscribed groups and estimating the Φ-statistics. In this way, the total genetic variation can be explained by showing the contribution of different data partitions: among groups (ϕ_{CT}); among populations within groups (ϕ_{SC}) and within populations (ϕ_{ST}).

3.2.5 Phylogeographic process

Mismatch distributions of pairwise differences between populations were generated with Arlequin v. 2.0 (Schneider et al., 2000) to test the null hypothesis of demographic expansion. A Poisson shape distribution is assumed for the model of demographic expansion, which in conjunction with Harpending's (1993) raggedness index is used to determine the statistical significance of the observed distribution.

Geographic association of population genetic variation and structuring was investigated with Nested Clade Analysis (NCA) (Templeton et al., 1987; Templeton and Sing, 1993; Templeton et al., 1995), run in Geodis v. 2.2 (Posada et al., 2000). Geographic association of haplotypes could be due to restricted gene flow, or historical demographic and vicariant events, or a mixture of both (Templeton et al., 1995). NCA nests haplotypes into clades on a network. Haplotypes separated by one mutation are nested (one-step clades), then these clades separated by one-mutation are nested (2-step clades) and so on, until a total cladogram is achieved. Nesting proceeds from the tips to the interior of the network. This nesting format, combined with the geographic distance between all populations sampled, allows the calculation of two clade distances: D_C, the average distance of all haplotypes within the clade from their geographic centre; D_N, the nested clade distance, where the average distance of all haplotypes of clade X are calculated in relation to the geographical centre of the nesting clade Y. A key is provided to interpret the differences observed between D_C and D_N (Templeton et al., 1995; updated on web).

3.3 Results

3.3.1 Sequence Analyses

A partial fragment of the mitochondrial cytochrome *b* gene was sequenced for a total of 708 individuals from 32 populations (Table 3.1). For *C. ornata* and *C. radians* a fragment of 328bp was amplified and for *C. flava* a 359bp fragment was amplified. A total of 54 different haplotypes were identified (Table A3.2.1; A3.2.5; A3.2.10). Tajima's D and Fu's Fs tests were both non-significant for *C. ornata* and *C. flava* (Table 3.2) but a significant result for *C. radians* rejects the null hypothesis of no selection for this species (Table 3.2), indicating that *C. radians* may not be in an equilibrium state due to a bottleneck event, selective sweep or direct natural selection.

Haplotype diversity (*h*; Table 3.2) ranged from 0.333 (*C. flava)* where one haplotype occurred in all populations at a frequency greater than 60% (Table A3.2.12) to 0.638 (*C. ornata*) where two dominant haplotypes occurred in 40% and 50% of the total sample (Table A3.2.3). The average nucleotide diversity (π; Table 3.2) was low for all three species (π = 0.002 - 0.008), suggesting a shallow phylogenetic structure within species.

Table 3.2. Summary of molecular analyses for three species of *Cellana* limpets

	C. ornata	*C. radians*	*C. flava*
N	302	321	85
No. of populations	31	31	8
No. of haplotypes	21	29	4
h ± S.D.	0.6375 ± 0.0171	0.4131 ± 0.0351	0.3325 ± 0.0562
π ± S.D.	0.007926 ± 0.004708	0.003907 ± 0.002726	0.001901 ± 0.001621
D (P)	-0.3 (> 0.10)	-1.74 (< 0.01)	0.27 (> 0.10)
Fs (P)	-4.47 (> 0.05)	-25.29 (< 0.01)	0.26 (> 0.05)
Haplotype divergence (%)	0.3 – 1.5	0.3 – 2.0	0.3 – 0.6

Divergence between haplotypes was similar across species, ranging from 0.3% to 2.0% (Table 3.2). However, the overall genetic structure of the species around Cook Strait differs (Fig. 3.2 a-c). An ancestral haplotype has given rise to separate *C. ornata* lineages, each of which has undergone subsequent radiation (Fig. 3.2a). These lineages are mostly restricted to the North (O7) and South (O1) Island (Fig 3.2a) with a low occurrence of sharing between the islands. The South Island haplotype is shared with three east coast populations of the North Island at frequencies of less than 10% (Table A3.2.3). The main North Island haplotype is shared only with French Pass on the northern coast of the South Island. Cape Kidnappers also shares haplotype O4 with South Island, east coast, populations. Haplotype O6 was the only other haplotype to be shared between the North and South Islands (Fig. 3.2a; CC, HAW) at a frequency of 10% in both populations (Table A3.2.3). This intermediary haplotype is indicative of an ancestral lineage.

In contrast, the characteristic star-shaped phylogeny of *C. radians* (Fig. 3.2b) shows an excess of rare haplotypes indicative of a demographic bottleneck with a subsequent range expansion. One main haplotype (R1) occurs in all populations (Table A3.2.7) with a further two haplotypes shared between the North and South Islands (Fig. 3.2b), like *C. ornata* these haplotypes occur in the east coast populations of the North and South Island, as well as French Pass and the southern most populations of the North Island. There are also five haplotypes shared amongst South Island populations and one haplotype shared within North Island populations only.

C. flava has two shared and two unique haplotypes (Fig. 3.2c) with a maximum of only two nucleotide substitutions between the most divergent haplotypes. A main haplotype is shared across all populations and a second shared haplotype occurs only in the populations of the North Island (Table A3.2.12).

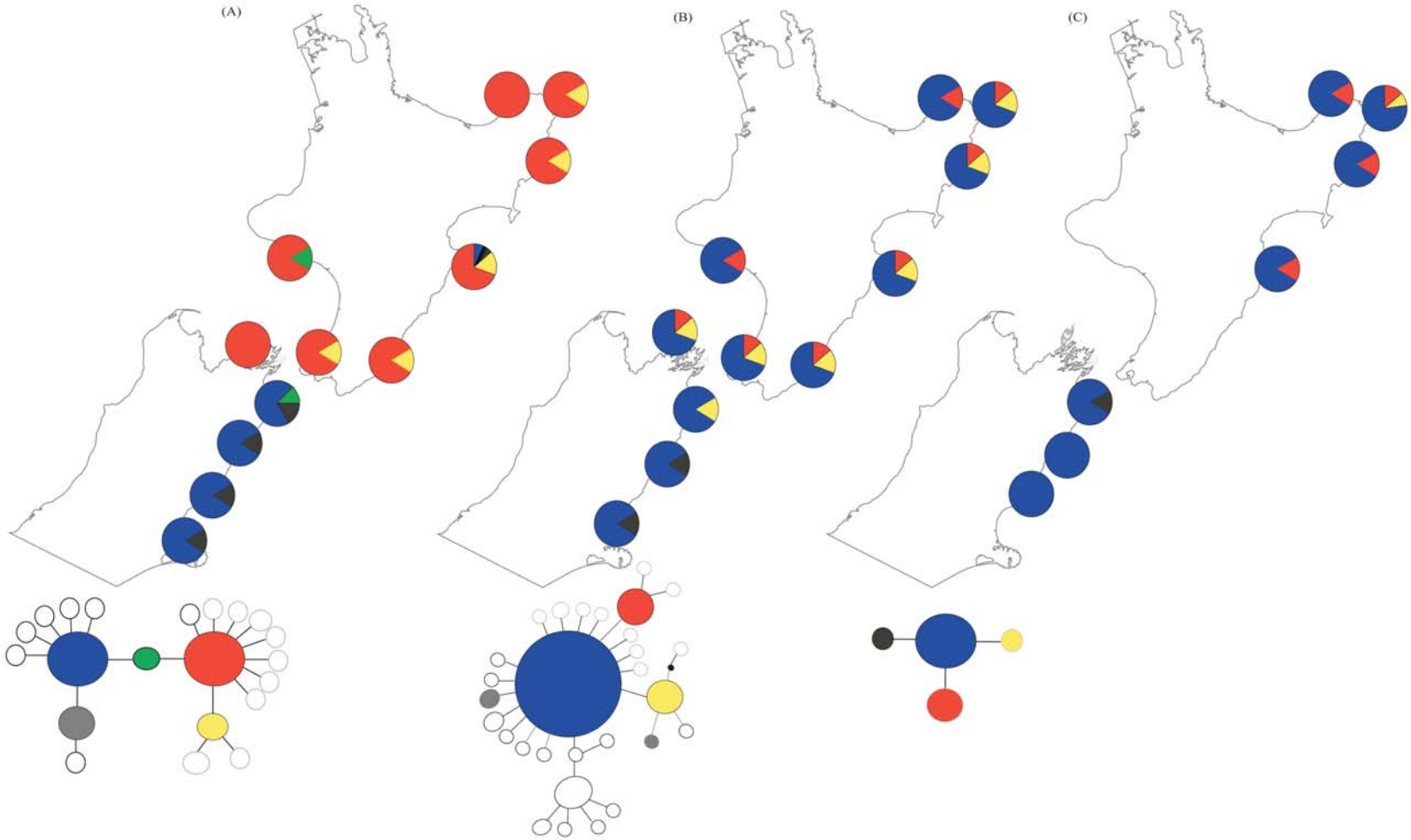

Figure 3.2. Statistical parsimony networks and the distribution of haplotypes shared between the North and South Islands for (a) *Cellana ornata*, (b) *C. radians* and (c) *C. flava*. Pie charts represent haplotype frequencies within populations for each species.

3.3.2 Phylogeographic Structure

A significant amount of genetic variation was explained by pooling North Island and South Island populations in all species. Multi-dimensional scaling (MDS) of d_{XY} (Appendix III) showed a clear division between North Island and South Island populations for *C. ornata*, although one population on the north coast of the South Island (French Pass (FP)) was tightly grouped with North Island samples. The nucleotide differentiation exhibited by *C. radians* and *C. flava* (Fig. 3.3B-C) is less clear due to the increased differentiation between populations of the South Island (*C. radians*) and the North Island (*C. flava*). As with *C. ornata*, *C. radians* samples from the French Pass population are tightly grouped with North Island populations, but for this species Cape Campbell, Kaikoura, Little Pigeon Bay, Dunedin and Stewart Island are also grouped with the North Island samples

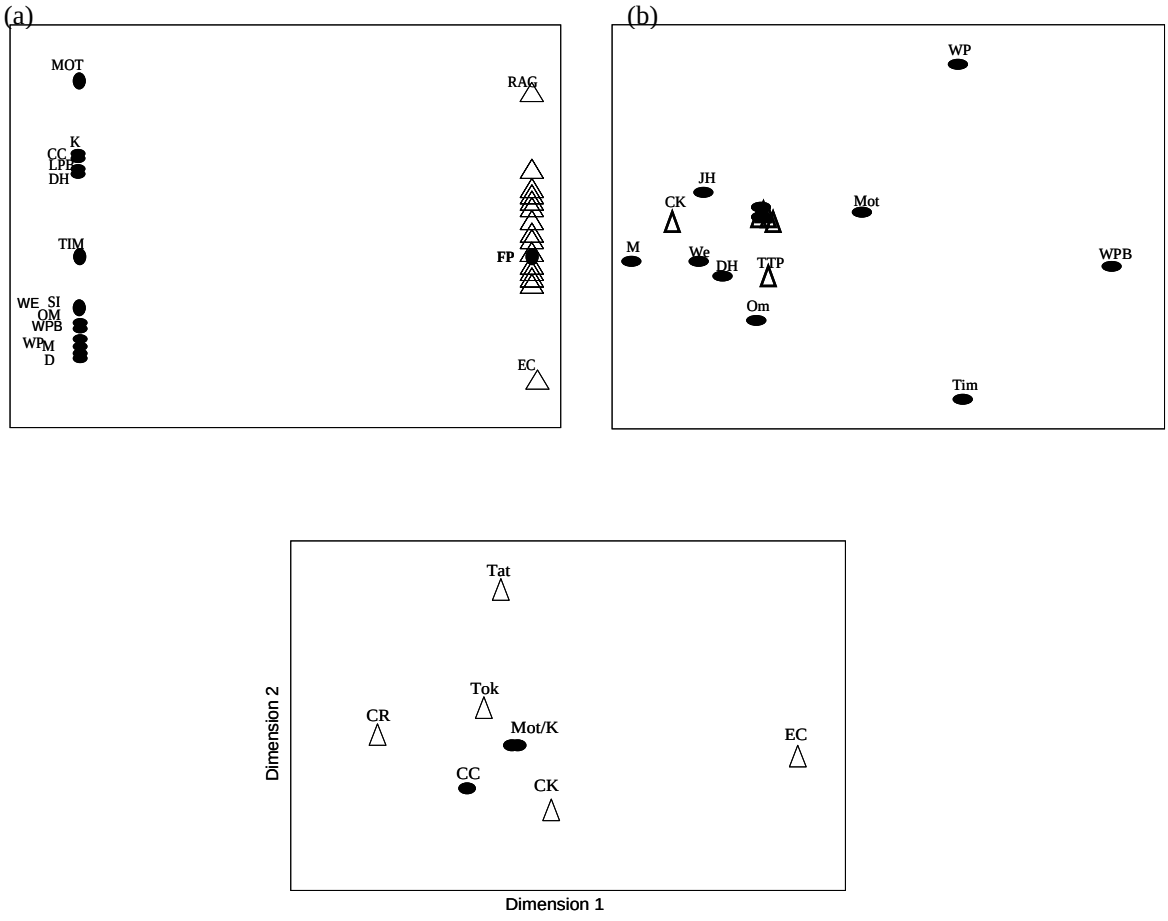

Figure 3.3. MDS plots of Nei's genetic differentiation between populations (d_{XY}; Nei, 1987) of (a) *Cellana ornata*, (b) *C. radians* and (c) *C. flava*. The stress value for all analyses (a-c) was < 0.005. Key: circles, South Island population; triangles, North Island populations. Populations codes are given in Table 3.1.

Analyses of Molecular Variation (AMOVA) were performed on the three species. Populations were partitioned into two groups, North Island (group 1) and South Island (group 2), revealing that a significant proportion of the total genetic variation was attributable to among group variance (Table 3.3). The variance was particularly high for *C. ornata* (76.63%), with Φ_{ST} (0.83) demonstrating a significant level of genetic structure within populations. *C. radians* and *C. flava* exhibited considerably less structure among groups, 4.6% and 17.7% respectively, with considerably lower Φ_{ST} values (0.14 and 0.18). In addition, there was significant variation within groups for *C. ornata* and *C. radians* but *C. flava* populations exhibited greater genetic variation within populations than between populations, which biased the variation within groups resulting in no differentiation between them.

Table 3.3. Φ-statistics from AMOVA of *C. ornata*, *C. radians* and *C. flava*, where North Island and South Island populations were partitioned into two groups for each species.

Source of Variation	Φ_{CT}	Φ_{SC}	Φ_{ST}
C. ornata	0.76631 **	0.27205 **	0.82988 **
C. radians	0.04630 **	0.10045 **	0.14210 **
C. flava	0.17689 **	-0.05830 **	0.17689 **

Note: AMOVA were run using pairwise nucleotide differences. Φ-statistics were estimated and tested with 1023 random permutations. ** $p < 0.01$ in Arlequin (Schneider et al., 2000).

A second AMOVA was run on *C. ornata* and *C. radians* populations (Table 3.4). In these AMOVA French Pass was pooled with the North Island samples due to the affinities this population showed with the North Island populations in both species (Fig. 3.3). The percentage of variation accounted for by the north-south split increased for both species with this alteration. Among group variation increased to 83.26 % for *C. ornata* and 5.71% for *C. radians* (Table 3.4). The variation among populations within groups was also reduced for both species: Φ_{SC} was reduced from 0.27 to 0.04 (*C. ornata*) and from 0.10 to 0.09 (*C. radians*), although the influence of this partition was still significant.

Table 3.4. Φ-statistics from AMOVA of *C. ornata* and *C. radians*, where French Pass was treated as a North Island population.

Source of Variation	Φ_{CT}	Φ_{SC}	Φ_{ST}
C. ornata	0. 83258 **	0.04067 **	0.93939 **
C. radians	0.04630 **	0.10045 **	0.14210 **

Note: AMOVA were run using pairwise nucleotide differences. Φ-statistics were estimated and tested with 1023 random permutations. ** $p < 0.01$ in Arlequin (Schneider et al., 2000).

3.3.3 Phylogeographic Process

The low genetic diversity and haplotype divergence of the *C. flava* data prevents further statistical investigation of processes predicting the observed phylogeographic pattern. Therefore, this section is focused on *C. ornata* and *C. radians* only.

Mismatch distributions of pairwise differences between populations were plotted to further investigate the rejection of neutrality in *C. radians* and to compare this with the expected equilibrium state of *C. ornata*. The observed mismatch distributions (Fig. 3.4) and raggedness indices are consistent with Tajima's and Fu's test of selection (Table 3.2). The Poisson shape expected by the model of sudden range expansion was observed for *C. radians* only (Fig. 3.4B; SSD = 0.087, $p > 0.1$). Demographic expansion is therefore accepted as a possible process shaping the genetic structure of *C. radians* populations (raggedness index, 0.476; $p > 0.1$) but not *C. ornata* populations (raggedness index, 0.406; $p < 0.01$).

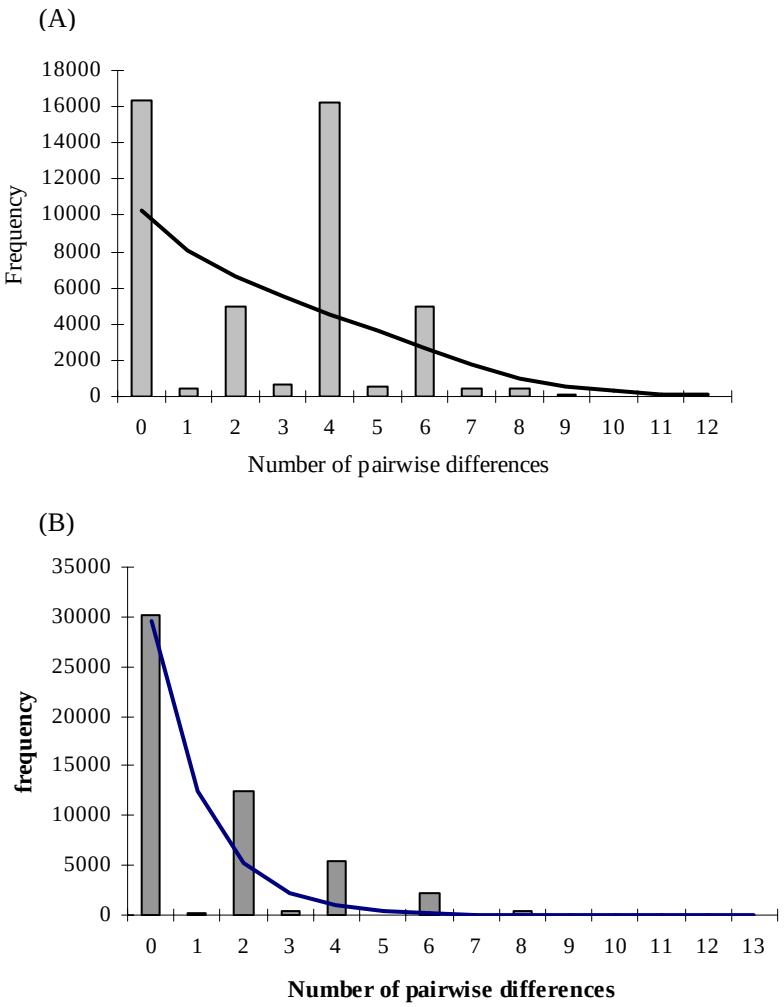

Figure 3.4 Distribution of pairwise differences among populations of (A) *C. ornata* (SSD = 0.136, $p < 0.001$) and (B) *C. radians* (SSD = 0.087, $p > 0.1$). The histograms represent the observed differences, while the lines represent the distribution expected under the model of range expansion. Population expansion is accepted for *C. radians*.

Nested Clade Analysis based on the Statistical parsimony network of *C. ornata* and *C. radians* (Fig. 3.5a-b) revealed a significant association between haplotypes (or higher level clades) and geographic distributions for both species (Table 3.5). The significant associations show a pattern of allopatric fragmentation between the North Island and the South Island for both species as well as subsequent range expansion for *C. radians* and restricted gene flow on the South Island for *C. ornata*. Allopatric fragmentation occurs in both species due to the partial non-overlapping geographic distribution of lower clades, restricted in distribution to the South Island, nested within higher clades of wide distribution.

Lower clades for *C. radians* also exhibit allopatric fragmentation due to the many rare haplotypes of restricted distribution.

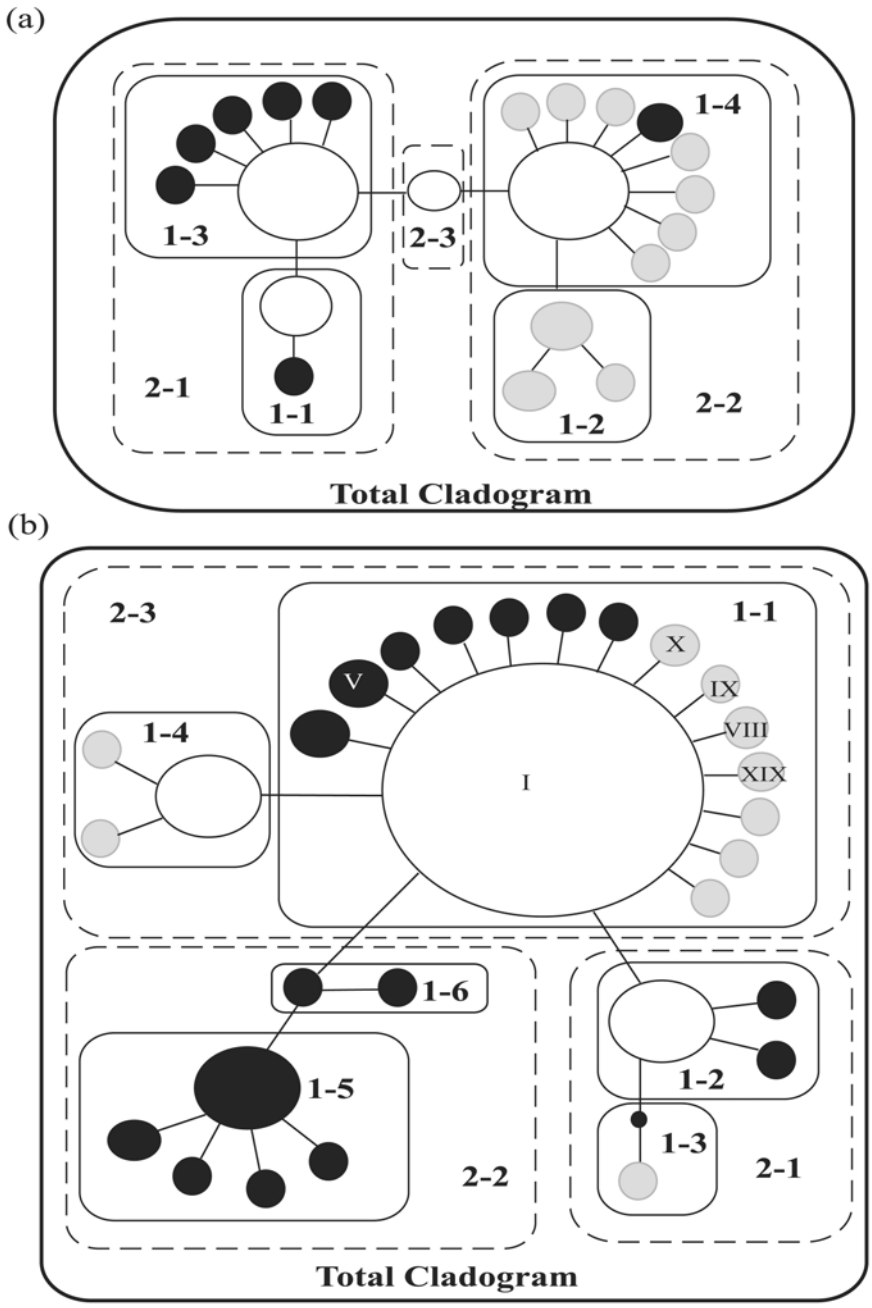

Figure 3.5. Statistical parsimony networks and associated nesting designs for (a) *Cellana ornata* and (b) *C. radians*. The area of the circles are proportional to the number of individuals sharing each haplotype (scale is consistent across all species). Branch connections represent one mutational step. Shading: dark, haplotypes unique to the South Island; light, haplotypes unique to the North Island; no shading, haplotypes shared between islands. The small black circle (b, clade 1-3) represents a hypothetical haplotype that was not observed. Border: Fine, single step clade (1-1 to 1-4); dashed, 2-step clades (2-1 to 2-3); Dark, total cladogram.

Table 3.5. Summary of Nested Clade Analysis results for (a) *C. ornata* and (b) *C. radians*.

(a) *C. ornata*			Total Clades: 9			
Significant clades	X^2	P	Subclades	Dc	Dn	Inferred Scenario
2-1	103.1	0.0060	1-1 (T)	194^{S}	361^{S}	Restricted gene flow within the South Island
			1-3 (I)	491^{L}	475^{L}	
			I-T	297^{L}	475^{L}	
Total Cladogram	283.61	0.0000	2-1 (T)	466^{S}	723	Allopatric fragmentation
			2-2 (T)	567^{S}	746	
			2-3 (I)	214	563	
			I-T	-305	-172	
(b) *C. radians*			Total Clades: 9			
Significant Clades	X^2	P	Subclades	Dc	Dn	Inferred Scenario
1-1	51.06	0.0420	I (I)	738^{S}	745^{S}	Allopatric fragmentation with subsequent range expansion.
			V (T)	551^{S}	794	
			VIII (T)	0	1052^{L}	
			IX	0	1052^{L}	
			X	0	1052^{L}	
			XIX	0	492^{S}	
			I-T	442	-60^{S}	
2-3	39.11	0.0140	1-4 (T)	500^{S}	680	Allopatric fragmentation
			I-T	259^{L}	76	
Total	141.53	0.0000	2-1 (T)	598^{S}	686	Allopatric fragmentation
Cladogram			2-2 (T)	590^{S}	762	
			I-T	159^{L}	30	

3.4 Discussion

The data obtained from the *Cellana* genus are consistent with the vicariant fragmentation of habitat around the Cook Strait region. The clearest example of this is *C. ornata*. Within a broad biogeographic range, *C. ornata* shows well-established northern and southern clades with radiation occurring within each island (Fig. 3.2). The phylogeographic structure is less obvious for *C. radians* than for *C. ornata* due to the fluctuation in population expansion observed for this species but is still statistically significant (Table 3.3 and Table 3.4). *C. flava* exhibits less

haplotypic diversity than that of *C. ornata* or *C. radians*, yet even for this species, a significant pattern of isolation is observed between North and South Island populations, as indicated by the occurrence of a frequent, but unique North Island haplotype (F3).

The timing of the fragmentation of each species is undetermined. This could reflect the effective population sizes of the different species at and during the period of fragmentation. Disparity in the demographic history between species can create inconsistencies in the rate of coalescence due to the reliance of the mutation rate on the effective population size (Harvey, 1996). A decrease in the effective population size increases the rate of coalescence, reducing the perceived time to the most recent common ancestor and increasing the number of recent mutations within the population (DeSalle and Templeton, 1988; Galtier et al., 2000). *C. flava* is restricted in its distribution, possibly reducing the effective population size of the species as a whole. *C. radians* exhibits demographic expansion, indicating a reduction in the effective population size in the history of the species.

In addition there is a reproductive disparity between the species. *C. ornata* is reproductively active during the late Summer (February – March) (Dunmore and Schiel, 2000), *C. flava* spawns in the early summer period (November – December) and *C. radians* has multiple releases throughout the year (Creese and Ballantine, 1983). The physical conditions at the time of larval release or spawning establish the initial conditions of larval survival, trait expression and dispersal direction (Sponaugle et al., 2002; Yamahira, 2004; Hendry and Day, 2005). Therefore, gene flow between populations may be restricted due to dispersal direction and larval survival variation based on the timing of release, creating disparities between species.

The influence of biogeographic fragmentation is often overlooked due to the long distance dispersal potential of the species being studied, such as echinoderms (Mladenov et al., 1997; Waters and Roy, 2004) and mussels (Smith, 1988; Apte and Gardner, 2002). This is especially true in New Zealand where the oceanographic characteristics are likely to strongly influence oceanic dispersal (Schiel, 2004). However, long distance dispersers are often found to maintain historical genetic structuring despite good dispersive currents. For example, stomatopods in the Indo-West Pacific exhibit high population genetic structure, which is thought to have

originated when marine transgression isolated ocean basins during the Pleistocene. This genetic structuring persists in spite of the potential for long distance dispersal in this region (Barber et al., 2002).

My data are consistent with several phylogeographic studies on coastal invertebrates of New Zealand. As for the echinoderms studied by Sponer and Roy (2002) all three *Cellana* species have shared-haplotypes along the east coast, north and south of Cook Strait and within Cook Strait. Similarly, Apte and Gardner (2002) showed evidence for a north-south split occurring in the green-lipped mussel *Perna canaliculus* to the south of Cook Strait. Although my data are consistent with the *Perna* data, the observed sharing of haplotypes suggests an alternative explanation.

Apte and Gardner (2002) reported that upwelling to the south of Cook Strait is acting as a barrier to dispersal of *Perna canaliculus* but they presented no evidence that an upwelling region is restricting gene flow. They had very few sample sites around the Cook Strait and upwelling region necessary to test for the possibility that upwelling is involved in the process of population structuring. More recently, Waters and Roy (2004) used an extensive sampling regime to study population structure of the sea-star *Patiriella regularis*, with the same process of upwelling invoked to explain the observed genetic population structure. Waters and Roy (2004) incorporated populations around the northern region of the South Island plus three southern North Island populations: Wellington, Opunake and Kairakau. However, pivotal populations such as Cape Campbell and Castle Point were not included in the study. Consequently their conclusion that upwelling is the most likely barrier to dispersal across Cook Strait may alter if populations within the region of inferred upwelling are sampled.

In contrast to the previous high dispersing species, Mladenov et. al. (1997) found no evidence of a genetic break between the North and South Islands in another long distance disperser, *Evechinus chloroticus*, using allozyme markers, which suggests that the upwelling barrier may not be as effective to some larvae as it is to others, or that the isolation of islands is indeed a result of vicariant association as shown by the *Cellana* data.

Differences in sampling regimes among similar molecular studies within New Zealand make it difficult to resolve whether the genetic breaks are occurring in the

same region. The use of different genetic markers also makes it difficult to assess concordance in the timing of isolation of the coastal fauna around the Cook Strait region, and therefore the processes influencing the structure of the coastal shore communities. A full review of marine molecular studies in New Zealand is given in Chapter V.

I have shown that a genetic discontinuity occurs between *Cellana* populations on the east coast of the North and South Islands. Mixing and gene flow within Cook Strait is also evident. Cape Campbell is an important population sample as it coincides with upwelling barriers used to explain genetic structure in this region, yet this site has not been sampled in earlier studies (Chapter V). In conjunction with populations around the upwelling regions, populations within Cook Strait are important in determining the gene flow between the North and South Islands within the Strait. French Pass is clearly a South Island population with connections to the North Island. Other studies also show populations within the Marlborough region that are genetically closer or exhibit haplotypes shared with the North Island (Sponer and Roy, 2002).

The presence of South Island dominated haplotypes occurring on the east coast of the North Island as far north as Cape Kidnappers suggests migration between the populations or homoplasy. While the major ocean currents around New Zealand are well characterised it seems that intermittent upwelling is possibly overstated as a means of isolating populations. An alternative to the isolating effects of the oceanic environment is the connectivity through migration between populations separated by historical events. In this instance, the geological history of Cook Strait, which only 15,000 years ago was no more than a bay, would be pivotal in the isolation of populations on the North and South Island.

Chapter IV

Phylogeography and Dispersal: Have Sea Surface Currents Structured New Zealand limpet populations?

The unique oceanographic characteristics of New Zealand provide the opportunity to test phylogeographic hypotheses of larval dispersal for coastal marine biota. The coastline can be divided into areas influenced by currents that might be expected to facilitate long distance dispersal, such as the Southland Current, and areas where more complex oceanography may cause localised retention or barriers to dispersal, such as the Wairarapa Eddy. Phylogeographic analyses were conducted on the mitochondrial cytochrome *b* DNA sequences of *Cellana ornata* and *C. radians* to assess the distribution of genetic variation between populations with respect to the major sea surface currents of New Zealand. 624 samples from 32 populations were analysed from throughout the biogeographic range of the two species. Phylogeographic analysis of these sequences revealed low, but significant, levels of genetic differentiation within and between designated current-zones. Genetic homogeneity was observed within zones that are influenced by constant along-shore currents such as the Southland Current zone, whereas the more complex oceanographic regions coincide with greater genetic differentiation and increased diversity for both species. However, historic association of the observed genetic structure with a once contiguous coastline cannot be ruled out.

4.1 Introduction

Dispersal in the marine environment is an important means of population connectivity and potential gene flow (Palumbi, 2003). The extent to which larvae are under the control of oceanographic factors during their dispersal phase has been a topic of great debate for many years (Butman, 1989). The transport of planktonic larvae in offshore currents is most likely to be influenced primarily by the prevailing currents. In the nearshore environment larvae must at some point enter the water column and at a later date settle out of the water column. This benthic-pelagic link is decoupled in time and space, suggesting that factors acting on the adults and the larval stages are important at a regional scale that covers an area as large as the mean dispersal distances of the species (Palumbi, 2003). However, both stages may also rely on the swimming ability and behaviour of the larvae or the larval characteristics such as size, density and spawn mass (Thiébaut et al., 1998). Many ecological, oceanographic and molecular techniques have been applied to understanding the role of physical factors on gene flow and larval dispersal (Gaines and Bertness, 1993; Largier, 2003; Palumbi, 2003).

Ecological and oceanographic modelling estimates of larvae involving capture and count methods have helped identify the roles of larval behaviour and oceanic circulation, but the extent to which larvae actively assist transport or are passive drifters is still not clear (Scheltema, 1971; Butman, 1989; Castilla and Varas, 1998; Todd, 1998; Castilla, 2001). Unfortunately, the larval stages of most intertidal organisms are not readily captured and despite much effort in this aspect of larval ecology, few studies have shown representative sampling of the larval pool necessary to test the influence of either currents or behaviour on larval transport. For example, intertidal traps were set on a rocky intertidal platform at Botany Bay, New South Wales, Australia, to collect larvae of the honeycomb barnacle *Chamaesipho tasmanica* (Jeffrey and Underwood, 2000). In this study the mean number of cyprids captured in sets of 3 traps was 0-5, with peaks of 15-60. It is difficult to know if such low numbers are representative of the larval pool or represent random capture due to fluctuating wave action. Therefore, realised dispersal within the intertidal environment remains unclear (Gaines, 1993).

Population genetic studies provide an indirect measure of dispersal potential through the examination of population connectivity and gene flow. Pioneering molecular studies utilising allozymes generally show the homogenous genetic distribution expected of marine organisms with high dispersal potential. For example, Apte and Gardner (2001) examined seven allozyme loci across 10 widely distributed populations of the mussel *Perna canaliculus* throughout New Zealand and observed no genetic differentiation between these populations. However, as molecular techniques have developed, previously undetected genetic structure in marine populations has been documented (Apte and Gardner, 2002). For instance, when Apte and Gardner (2002) re-evaluated the genetic structure of *Perna canaliculus* with mitochondrial DNA, genetic discontinuities were observed between populations. It seems that marine organisms previously thought to be capable of homogenising dispersal, rarely conform to Wright's island model (Wright, 1969), which assumes equal migration between populations (Wares et al., 2001).

In New Zealand, ecological, oceanographic and molecular techniques have been applied to address the issue of larval transport within and across regions influenced by oceanic currents. In an early study using aerial photography, large aggregations of the pelagic decapod *Munida gregaria* were observed at a headland front off of the coast of the Otago Peninsula (Zeldis and Jillet, 1982). Laboratory experimentation helped to confirm that the aggregations were a result of positive phototaxis and the converging of oceanic and estuarine water around the headland (Zeldis and Jillet, 1982). Similarly, Murdoch (1989) reported that the Otago headland eddy (Blue Skin Bay Eddy, Fig. 4.1) was not only carrying pelagic organisms onshore but was retaining eggs and larvae of inshore fish and crustaceans. Further north, Murdoch et al. (1990) collected eggs and larvae of Hoki (*Macruronus novaezelandiae*) in Cook Strait and determined that the distribution of the eggs was a result of advection from local upwelling in the Cook Strait Canyon transporting the eggs into the nearshore region off Cape Campbell. Chiswell and others (Chiswell and Roemmich, 1998; Chiswell and Booth, 1999) have done extensive oceanographic surveys of the currents and circulation in the north-eastern waters off New Zealand, including the investigation of larval retention within the eddies of this region. This work focused on the larvae of the rock lobster *Jasus edwardsii*, which they found to

be retained within the large Wairarapa Eddy (Fig. 4.1). They concluded that the gradient of larval stages found shoreward within the eddy was influenced first by passive transport and later by shoreward swimming of older larvae.

The physical oceanography of New Zealand was reviewed by Heath (1985) using data from 1970. The characterisation of the major sea surface currents from this review (Fig. 4.1) has become the basis of population genetic studies in New Zealand waters.

In 1989, regional genetic variation in electromorph frequencies in the tuatua *Paphies subtriangulata* around New Zealand showed two mainland geographic groups (Smith et al., 1989). It was reported that the East Auckland, East Cape, Tasman, D'urville and Southland currents (Fig. 4.1) were maintaining the isolation of these populations through directional transport. Similarly, allozyme variation in the mussel *Perna canaliculus* showed isolation of the northern populations of the North Island, which Smith (1988) suggested was partially influenced by the direction of the currents and partially by genetic-physiological thermal adaptation. In contrast, more recent molecular studies on *P. canaliculus* (Star et al., 2003) using RAPD techniques, found no significant genetic structure throughout the North Island. They did observe genetic structure within the South Island, separating the east and west coast populations. Allozyme data for *Evechinus chloroticus* showed connectivity of all populations throughout New Zealand, providing no evidence for structure by the major currents and suggesting nearshore stepping-stone migration (Mladenov et al., 1997). Molecular studies reporting a genetic discontinuity between the North and South Islands also suggest the influence of upwelling and tidal circulation as a process isolating populations (Apte and Gardner, 2002; Waters and Roy, 2004).

Many oceanographic features exist that are transient in nature. These anomalies include eddies, fronts and wind-induced variation in current flow. For example, the west coast of the South Island is characterised by a northward flowing Westland Current (Heath, 1985). However, it is debated whether the Westland Current and the associated upwelling around Cape Farewell (Fig 4.1) are persistent features of the coastal waters (Greig et al., 1988), or are wind-induced and therefore dominant only when a persistent onshore wind is blowing (Shirtcliffe et al., 1990). Banks Peninsula on the east coast (Fig. 4.1) has also been identified as a possible

upwelling area during a sustained period of north to north-westerly winds (McKendry et al., 1988).

Further north around Kaikoura and Cape Campbell the influence of nearshore currents is less clear. The Southland Current is always depicted as heading east at either Banks Peninsula and/or Kaikoura (Heath, 1985; Apte and Gardner, 2002), although Heath (1972) reported a strong northward flow of surface water during all seasons in this area. This region is also subject to regular intrusion of warm water patches first noted by Charles Fleming in 1952 (Heath, 1972; Chiswell and Roemmich, 1998), which are now characterised as water from the East Cape Current being transported southward in an offshoot eddy to the Cape Campbell and Kaikoura regions, eventually deflecting offshore (Heath, 1972; Vincent et al., 1991; Chiswell and Roemmich, 1998; Uddstrom and Oien, 1999; Shaw and Vennell, 2000).

The aim of this chapter was to test a phylogeographic hypothesis, that the population genetic structure observed in *Cellana* species is concordant with the major sea surface currents around New Zealand.

C. ornata and *C. radians* were used in this study as they exhibit concordant biogeographic distributions and both species were identified in chapter III as exhibiting a genetic break around the Cook Strait region. I first examine the population structure of the two species to identify concordance with the major sea surface currents around New Zealand. For this I have partitioned the populations to match seven "current-zones" (Fig. 4.2). These current-zones depict coastal populations that occur within a region adjacent to the major sea surface currents (Fig. 4.1) as characterised by Heath (1985). Due to disparity in the reported behaviour of the Southland Current around Banks Peninsula I have investigated two groupings for populations on the South Island. In the first instance I have assumed that the Southland Current proceeds in a northward direction past Banks Peninsula to Cook Strait (Model A, Fig. 4.2). In a second model I have allowed deflection of the Southland Current at Banks Peninsula and have grouped all populations north of Banks Peninsula separately from the southern populations (Model B, 1b Fig. 4.2). In further analyses I investigated the correlation between mean annual sea surface temperature and population differentiation and haplotype frequency. A pairwise test of migration (Nielson and Wakeley, 2001) which allows for asymmetric migration

and differences in the effective population size between populations was used to investigate gene flow across Cook Strait and on the east coast of the North Island.

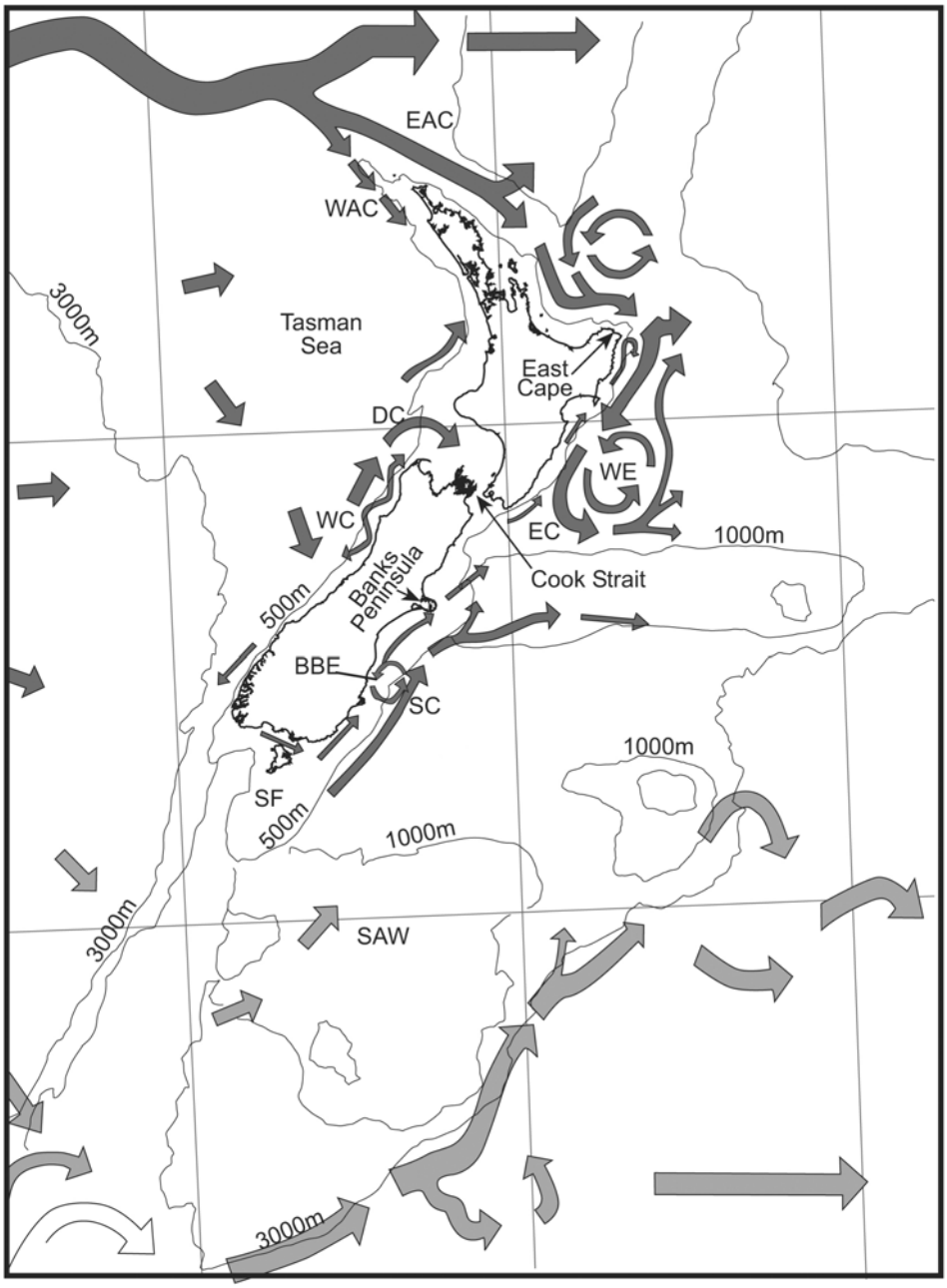

Figure 4.1. The currents and continental shelf around New Zealand. Abbreviations: SC, Southland Current; SF, Southland Front; WE, Wairarapa Eddy; EAC, East Auckland Current; WC, Westland Current; WAC, West Auckland Current; EC, East Cape Current. Redrawn from a map by the National Institute of Water and Atmospheric Research.

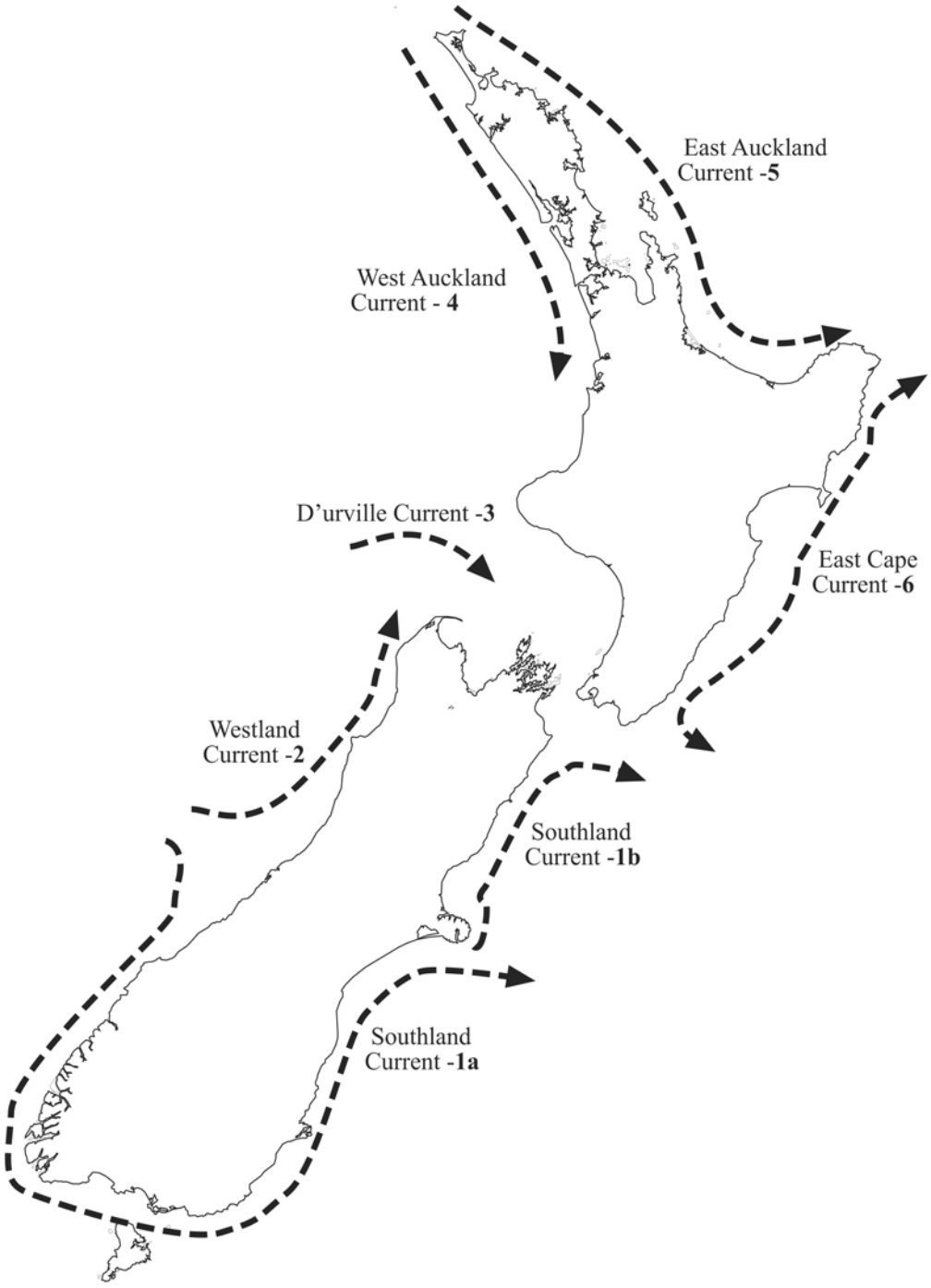

Figure 4.2. Map of New Zealand's major sea surface currents after Heath (1985). Numbers 1-7 identify the current-zones used for Analysis of Molecular Variance statistics in this study.

4.2 Materials and Methods

4.2.1 Sample Preparation

C. ornata and *C. radians*, were collected as described in Chapter III. These two species were chosen for this study due to their co-habitation and concordance of biogeographic range around the coast of New Zealand (Fig. 4.3). This concordance allowed the partitioning of the data set into distinct zones based on the positioning of populations adjacent to sea surface currents (Fig. 4.2). The DNA material and sequence data were also prepared as described in Chapter III.

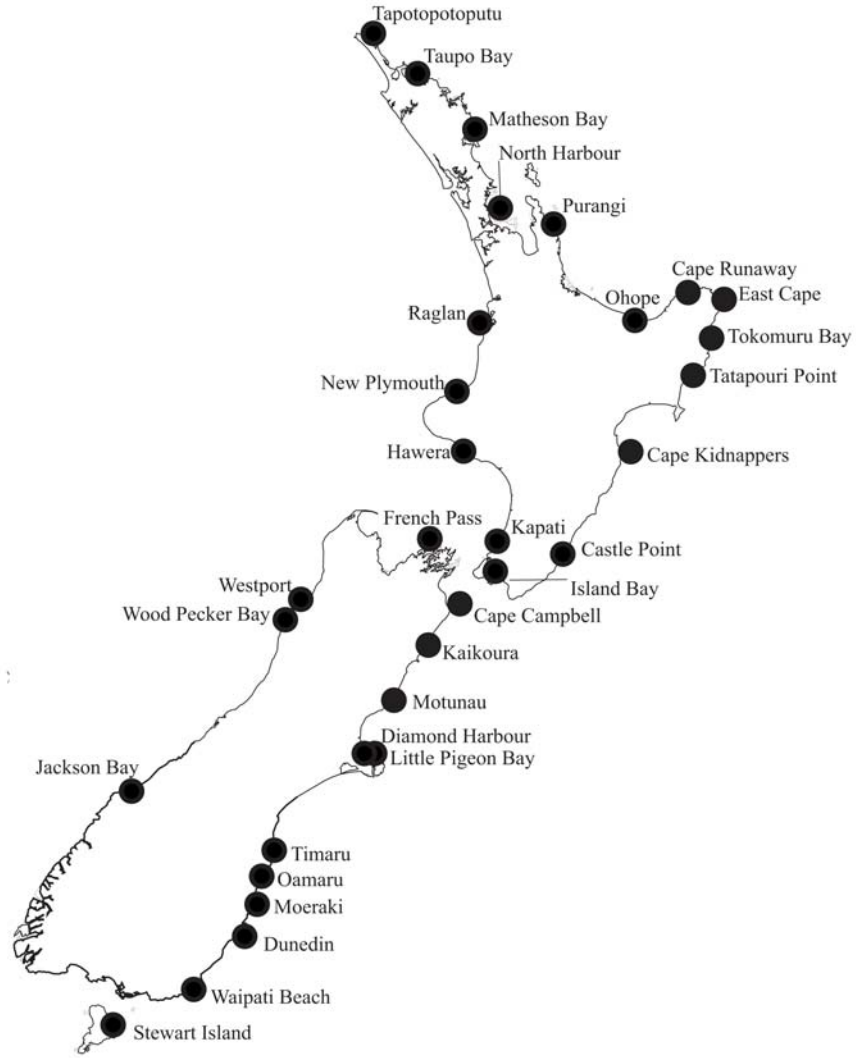

Figure 4.3. Study sites around New Zealand. Filled circles show the locations of samples used in this study. *C. radians* only was collected from Topotopotoputu. Only *C. ornata* was sampled from Taupo Bay.

4.2.2 Sequence Analysis

Genetic analyses for this chapter were conducted separately on the South Island and North Island populations due to the genetic discontinuity observed in Chapter III. The west coast populations were omitted in the later correlative analyses because of the low number of sample locations from this region.

Nei's (1987) nucleotide diversity (π) and gene diversity (h) were calculated using Arlequin v. 2.0 (Schneider et al., 2000). These indices are simple heterozygosity measures at the nucleotide level that do not depend on sample size and do not assume models of population dynamics (Nei and Kumar, 2000). They are deemed most appropriate for a single locus, of low diversity, where populations are not assumed to be in equilibrium (Chapter III). Statistical significance between current-zones was tested with t-tests, using a Bonferroni adjustment of alpha (α = 0.001) to account for multiple testing (Bland and Altman, 1995).

4.2.3 Phylogeographic Structure

Nei's (1987) corrected measure of nucleotide differentiation between populations (d_A) was calculated in Arlequin v. 2.0 (Schneider et al., 2000). This is a simple measure of genetic distance between populations based on the number of nucleotide differences between haplotypes present in each population. Within-population variation was used to correct the variation between populations (Nei and Kumar, 2000). Hypotheses of population genetic variation were tested by Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) in Arlequin v. 2.0 (Schneider et al., 2000). The *a priori* partitioning of data was defined with respect to the major sea surface currents around New Zealand (Fig. 4.2) producing 7 "current-zones": 1a, Southland Current north to Banks Peninsula; 1b Southland Current north of Banks Peninsula; 2, Westland Current; 3, D'urville Current; 4, West Auckland Current; 5, East Auckland Current; 6, East Cape Current.

The demographic expansion evident in *C. radians* (Chapter III) was explored at the population level. Mismatch distributions of pairwise differences between populations were run in Arlequin v. 2.0 (Schneider et al., 2000) to test the null hypothesis of demographic expansion within populations. A Poisson-shaped distribution was assumed for the model of demographic expansion. Harpending's

(1993) raggedness index and the Sum of Squared Deviations test (SSD) were used to determine the statistical significance of the observed distribution.

Mantel tests were performed using Arlequin v. 2.0 (Schneider et al., 2000) to examine the correlation between the distribution of genetic variation and the geographic distance between populations within each of the current-zones. If the currents are influencing linear dispersal of these species then isolation-by-distance would be expected. Isolation-by-distance was also examined along the entire east coast of New Zealand for both species. East coast samples were used due the extensive sampling regime in this area allowing a well distributed and meaningful test to be conducted. West coast samples were not used as the sampling along these coastlines was disjunct in many places.

4.2.4 Sea Surface Temperature

Partial correlations (Mantel, 1967) of mean annual sea surface temperature (SST) with genetic differentiation between populations and haplotype frequency (for *C. radians* only) were performed in Arlequin v. 2.0 (Schneider et al., 2000) to investigate the influence of abiotic factors, which are often correlated with SST, on the observed distribution of genetic variation as an alternative to dispersal by ocean currents.

The mean offshore annual SST for the period January 1993 to December 1997 was used for temperature correlations (Uddstrom and Oien, 1999). Uddstrom and Oien's (1999) off-shore temperature data were ground-truthed with coastal onshore data from Leigh, Auckland, Napier, Lyttelton, Timaru and Otago Peninsula (Greig et al., 1988). There was a negligible difference of c.0.5^{o}C at sites between the onshore and offshore data and so the offshore data was considered a good representation of SST for coastal organisms.

4.2.5 Migration

The method of Nielsen and Wakeley (2001) can be applied to single-locus, non-recombining sequence data to determine whether short divergence times and little migration or long divergence times and strong migration explain the pattern of genetic differentiation observed in a data set. This method is based on divergence between

two populations arising from a single ancestral population and allows for asymmetric migration and unequal population size. Three parameters are estimated by pairwise calculations, using an infinite-sites model of nucleotide substitution: theta (θ), a measure of genetic diversity approximated by twice the effective population size multiplied by the mutation rate ($2N_e\mu$); M, the proportion of the population replaced by migrants from another population, per generation; T, the coalescent rate of population divergence, or the generation time divided by twice the effective population size before divergence ($t/2N_1$). The parameters are estimated using a Markov Chain Monte Carlo method to calculate posterior distributions, with the assumption of uniform prior distributions: all possible values of the parameters are assumed to be equally likely before observing the data (Nielsen and Wakely, 2001). These analyses were done on-line through MDIV@ CBSU, using a chain length of 5,000,000 cycles and a burn-in time of 500,000 cycles. Mmax was set to 10 and Tmax set to 5; θ was estimated. These default parameters were altered where low resolution of probability was observed.

This pairwise analysis of migration and isolation was performed on the east coast populations. In the first instance I tested pooled samples of populations north and south of Cook Strait with the expectation of short divergence times and minimal to zero migration across the observed genetic barrier (chapter III). In a second analysis I focused on populations along the east coast of the North Island (CP, CK, Tat) to compare migration between these populations with no significant genetic differentiation and the more structured populations across Cook Strait (Chapter III).

4.3 Results

4.3.1 Sequence Analysis

A partial fragment of the mitochondrial cytochrome *b* gene was successfully sequenced for a total of 624 individuals from 32 populations (Table 3.1). A total of 50 different haplotypes (21 *C. ornata*, 29 *C. radians*) were identified (Table A3.2.1; A3.2.5).

Nei's (1987) nucleotide diversity (π) and gene diversity (*h*) indices revealed a low level of diversity throughout the range of *C. ornata* and *C. radians* (Fig. 4.4): 12 of 31 *C. ornata* populations exhibited no diversity while only 3 of the 31 *C. radians* populations were fixed. After a Bonferroni adjustment ($\alpha = 0.0011$) there was no significant difference in nucleotide or gene diversity between the current-zones for *C. radians*. In contrast, *C. ornata* shows significantly greater nucleotide diversity in current-zone 1b than in 1a and zone 5 is significantly less diverse than zones 1b and 4 (Fig. 4.4a). The gene diversity of zone 1b is also significantly greater than 1a and 5. Zone 6 exhibits significantly greater gene diversity than zone 5.

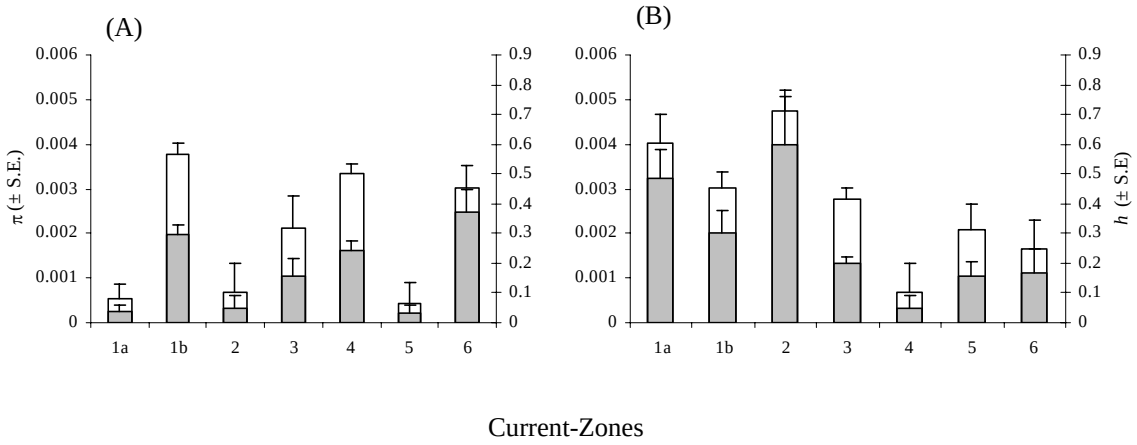

Figure 4.4. Nei's (1987) nucleotide diversity (π) and gene diversity (h) for current-zones of *C. ornata* (A) and *C. radians* (B). Only populations exhibiting diversity are shown. Grey bars represent π; outlined bars represent *h* .

4.3.2 Phylogeographic Structure

Haplotype sharing between current-zones is evident in both species (Fig. 4.5). One main haplotype (R1) is shared throughout all *C. radians* populations. In contrast, *C. ornata* has two main haplotypes that distinguish between North Island (O7) and South Island (O1) populations. Current-zone 6 on the North Island is unique in *C. ornata* in that it carries the South Island haplotype (O1) and a second east coast haplotype (O4) shared between zones 1a and 1b. *C. radians* has only one haplotype of five shared among South Island populations that is restricted to western and southern populations of zones 2 and 1a. A single haplotype is also restricted to the east coast of the South Island (1a and 1b). Concordance between *C. ornata* and *C. radians* is only apparent through the sharing of haplotypes in zones 1a and 1b. However, haplotype sharing within the zones was not concordant between the species.

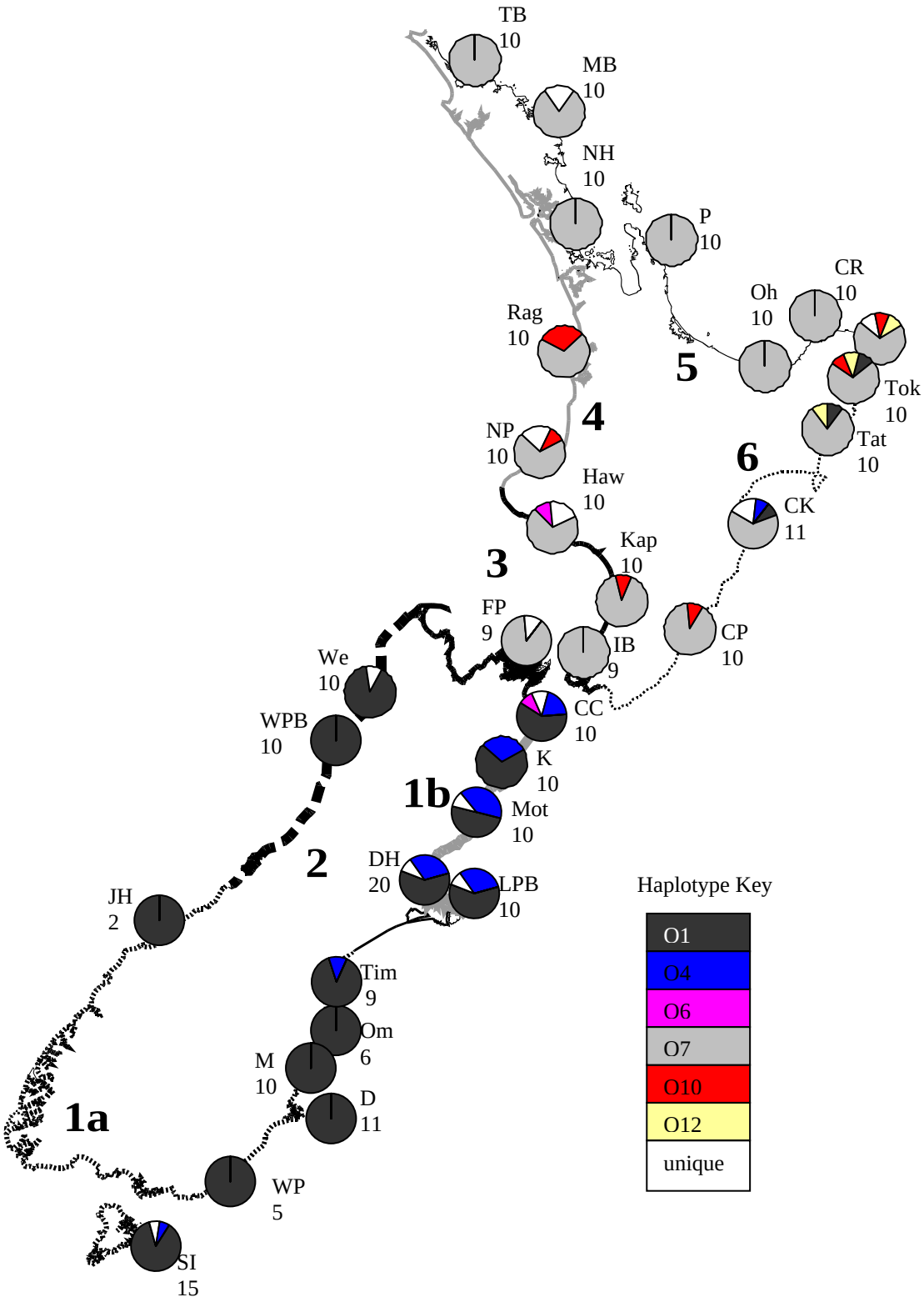

Figure 4.5a. Distribution of 21 shared and unique cytochrome *b* haplotypes throughout the range of *C. ornata*. Unique haplotypes are shown but are not labelled. Population codes and samples sizes are given. The haplotype key shows the colour used for each of the shared haplotypes represented. The outline of New Zealand depicts the seven current-zones (1a - 6) used for analyses.

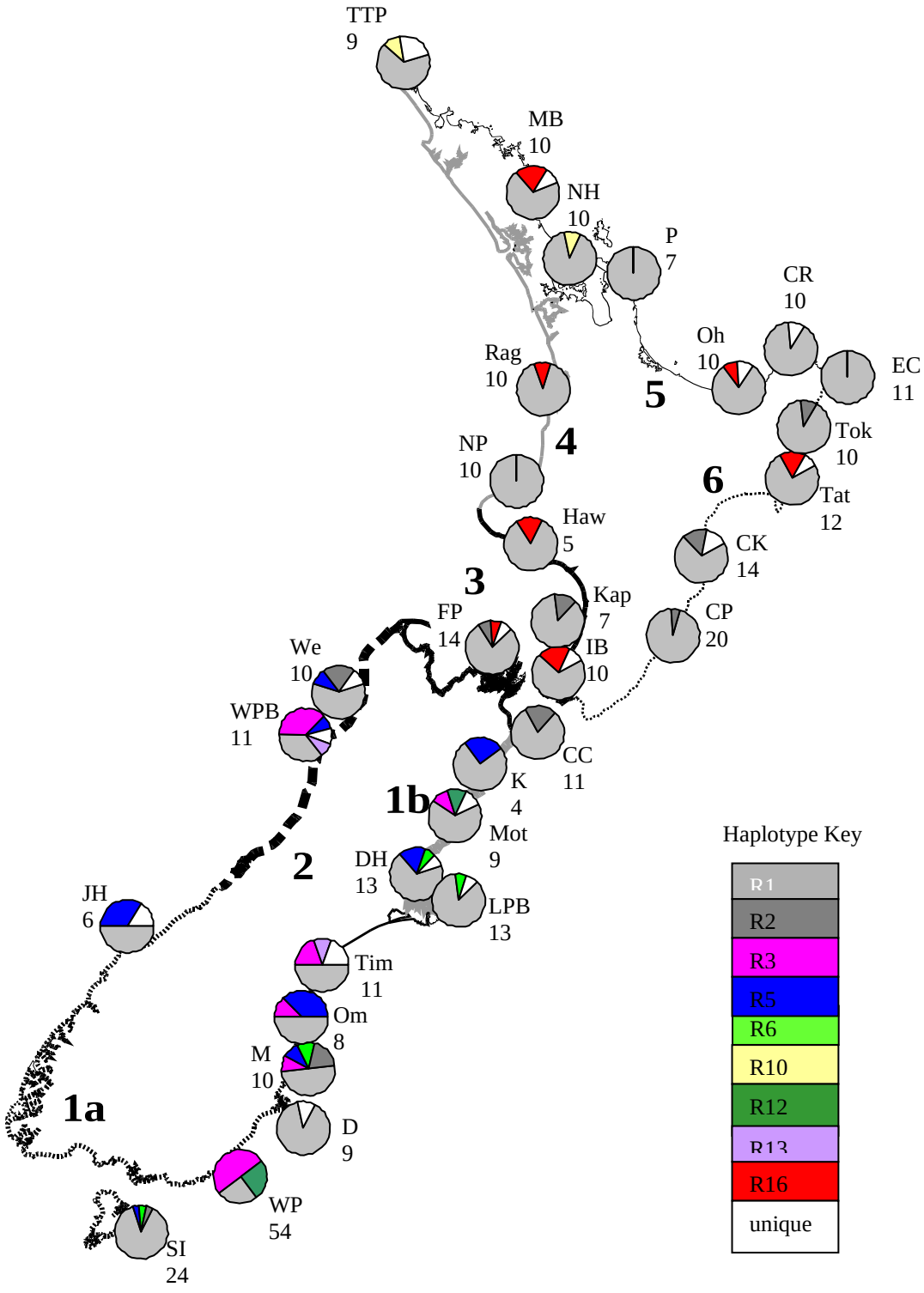

Figure 4.5b. . Distribution of 33 shared and unique cytochrome *b* haplotypes throughout the range of *C.radians.* Unique haplotypes are shown but are not labelled. Population codes and samples sizes are given. The haplotype key shows the colour used for each of the shared haplotypes represented. The outline of New Zealand depicts the seven current-zones (1a - 6) used for analyses.

The genetic distance between populations (d_A) was low for both species (Table A3.2.4 ;A3.2.8) ranging from 0.0 – 0.9 for *C. radians* and 0.0 – 0.2 for *C. ornata*. There was no significant genetic differentiation between populations within current-zones.

A series of AMOVAs run on both species revealed a small but significant contribution of current-zones to the total genetic variation within each species. Initial partitioning of the data followed the seven designated current zones (Fig. 4.2), although the North and South Island zones were treated separately (Table 4.1 – 4.4)

The variance among-groups (Φ_{CT}) on the North Island was low at only 5.12% for *C. ornata* and zero for *C. radians* (Table 4.1). The negative value of Φ_{CT} (-0.0077) for *C. radians* indicates that genetic variation within the zones is greater than the genetic variation among them, essentially cancelling any among group variation (Nei and Kumar, 2000). The low genetic variation between populations (Φ_{ST} = 0.04074 and 0.02259), confirms the weak genetic structuring within the North Island for both species. Negative variance is also observed within-groups (Φ_{SC}) of *C. ornata*, again this represents zero structure among-populations within-groups.

Table 4.1. Φ-statistics from AMOVA of two *Cellana* limpet species, where North Island populations have been pooled into 4 designated current-zones.

Source of variation	Φ_{CT}	Φ_{SC}	Φ_{ST}
C. ornata	0.05122**	-0.01105	0.04074**
C. radians	-0.00777	0.03012 **	0.02259**

Note: AMOVA were run using pairwise nucleotide differences. Φ-statistics were estimated and tested with 1023 random permutations. ** $p < 0.01$ in Arlequin (Schneider et al., 2000).

Two alternative models were proposed for the South Island. Using the first model, which combines zones 1a and 1b, AMOVA shows a significant proportion of the total variance attributed to the partitioned groups for both species (Table 4.2), albeit only 4% - 5%. The variation among populations within groups is of equal importance in *C. ornata* (Φ_{SC} = 0.05) but contributes significantly more in *C. radians* (Φ_{SC} = 0.12). Moderate genetic structure is suggested by the fixation indices (Φ_{ST}) which are 0.09559 and 0.15790 for *C. ornata* and *C. radians* respectively.

Table 4.2. Φ-statistics from AMOVA of two *Cellana* limpet species, where South Island populations have been pooled into 4 designated current-zones: Model A.

Source of variation	Φ_{CT}	Φ_{SC}	Φ_{ST}
C. ornata	0.04740**	0.05059**	0.09559**
C. radians	0.04606**	0.11724**	0.15790**

Note: AMOVA was run using pairwise nucleotide differences. Φ-statistics were estimated and tested with 1023 random permutations. ** $p < 0.01$. Model A: ((1a,1b),2).

The second model assumes that the Southland Current deflects offshore at Banks Peninsula (Fig. 4.2), and so the partitions were altered to include zones 1a, 1b and 2 separately. In these analyses (Table 4.4) the among-group genetic variation increased for *C. ornata* (5.2%) but was reduced for *C. radians* (Φ_{CT} = -0.00216). As a result of this, the within group genetic variance was reduced for *C. ornata* (Φ_{SC} reduced from 0.05059 to 0.03779) and increased for *C. radians* (Φ_{SC} increased from 0.11724 to 0.13000).

These results show that where *C. ornata* exhibits genetic discontinuity north and south of Banks Peninsula, a similar break is not observed for *C. radians*.

Table 4.3. Φ-statistics from AMOVA of two *Cellana* limpet species, where South Island populations have been pooled into 4 designated current-zones: Model B.

Source of variation	Φ_{CT}	Φ_{SC}	Φ_{ST}
C. ornata	0.05165**	0.03779**	0.08749**
C. radians	-0.00216	0.13000**	0.12813**

Note: AMOVA was run using pairwise nucleotide differences. Φ-statistics were estimated and tested with 1023 random permutations. ** $p < 0.01$. Model B: (1a, 1b, 2).

Mantel tests were performed within each of the current-zones. If the currents are influencing linear dispersal of these species then isolation-by-distance would be expected. No significant correlations were observed for any of the zones in either species. This suggests that the geographic distance covered by the currents is not greater than the potential dispersal distance of *C. ornata* or *C. radians*, or alternatively linear dispersal is not driving the genetic differentiation between populations. Similarly, isolation-by-distance was not observed along the entire east coast of the North Island or the South Island, for either species.

The Poisson shape distribution of pairwise differences between populations observed in Chapter III suggested that *C. radians* has experienced a demographic

expansion (Chapter III). The mismatch distribution and raggedness index (Harpending, 1993) for each population (Table A3.2.9) supports demographic expansion in all but five of the populations. The results for Woodpecker Bay, Timaru, Moeraki, Jacksons Head and Stewart Island were all significant, which suggests that these populations are in a stationary state. This leads to the rejection of the hypothesis of demographic expansion for these populations.

4.3.3 Sea Surface Temperature (East Coast)

The distribution of nucleotide differentiation between east coast populations for both species showed a significant association with the gradient of mean annual SST: *C. ornata*, $r = 0.801$; $p < 0.01$; *C. radians* $r = 0.129$ $p < 0.01$. In contrast, there was no correlation between SST and the frequency of the main *C. radians* haplotype (R1; $r = 0.022$, $p > 0.05$). The low genetic and temperature differences within islands on the east coast prevents similar correlations being done on these populations that would rule out bias in the data due to the north-south discontinuity at Cook Strait.

4.3.4 Migration

Pairwise estimates of migration and isolation were performed to investigate and contrast migration across Cook Strait and among populations of the eastern North Island. The method by Nielson and Wakely (2001) was examined as an alternative to Fst-based measures of migration due to the low sample size, low diversity and single locus characteristics of the two data sets. Posterior distributions of migration and divergence time were estimated for each species. However, in order for confidence limits to be calculated around the most probable value, upper and lower bounds (the probability of achieving a value greater than or less than the maximum) are required. The posterior probability distributions for both migration (M) and time since divergence (T) were only resolved with upper and lower bounds in one out of eight analyses performed on *C. ornata* and *C. radians* (Fig. A3.2.1; Fig A3.2.2). Without resolution in the upper boundaries, confidence levels cannot be assigned to the test and the distinction between migration and isolation cannot be inferred.

The analyses of pairwise estimates showed that minimal migration of *C. ornata* across Cook Strait does occur. Castle Point (CP) and Cape Kidnappers (CK) from the

North Island and Cape Campbell (CC) and Kaikoura (K) from the South Island were pooled for this analysis. The posterior probability distributions for M and T (Fig. 4.6) suggest low levels of migration with a long time since divergence between the North and South Island. The posterior probability distribution for M has a maximum at 0.2, suggesting that an average of 0.2 female individuals migrate cross Cook Strait, per generation. The posterior probability of T has a maximum of 2. However, as the upper limit of the distribution does not reach zero it can only be concluded that there is no evidence of a recent divergence and the two islands diverged at least 2 times 2Ne generations ago.

A population size of approximately 10,000 limpets may be inferred for the pooled South Island sample, based on a density of approximately 20 limpets m^{-2} (Dunmore and Schiel, 2003), across two platforms (Kaikoura and Cape Campbell) providing an area of approximately $1000m^2$. Assuming a 1:1 sex ratio, Ne may be estimated at approximately 12,000 individuals. Therefore, with a generation time of one year (Dunmore and Schiel, 2000) the divergence time may be estimated to at least 24 thousand years ago, although this is likely to be an overestimation.

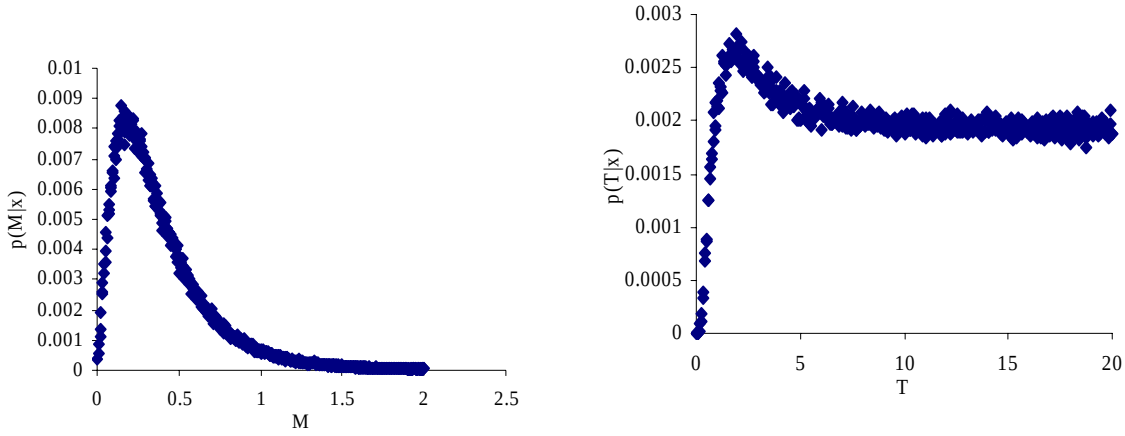

Figure 4.6. Posterior distributions of M and T for *C. ornata* pooled populations, north and south of Cook Strait. The distributions were generated under an infinite-sites model. A Markov chain length of 5×10^6 was used with a burn-in time of 5×10^5. Mmax was set to 2 and Tmax was set to 20; $\theta = 0.8$

4.4 Discussion

The results presented for *C. ornata* and *C. radians* suggest that contemporary 'current-zones' account for a small but significant proportion of the genetic structure for these species.

C. ornata and *C. radians* were chosen for this study because they have concordant biogeographic distributions and a short larval period. It was expected that these closely related species would exhibit concordant phylogeographic structure that would help to determine the influence of present-day sea surface currents on the genetic structuring of coastal populations. However, the results highlight the variability of genetic structure within a genus and suggest that contemporary processes cannot fully explain the genetic structure of these intertidal organisms.

Both species exhibit low genetic diversity ($\pi < 0.005$). However, the distribution of genetic diversity and therefore genetic differentiation varies greatly between the species. AMOVA results show that where current-zones of the North Island appear to contribute significant genetic structure to *C. ornata*, they have no observable impact on the genetic structure of *C. radians*. In the South Island, the same is true; *C. ornata* shows a genetic break between the southeast and northeast populations, whereas *C. radians* shows greater genetic distinction between the east and west coast populations.

These data are partially supported by other studies of marine organisms in New Zealand. *C. ornata* data shows a genetic discontinuity occurring around the region of Banks Peninsula. A genetic discontinuity in this region was also observed within mitochondrial lineages of the brittle star *Amphipholis squamata* (Sponer and Roy (2002). Sponer and Roy (2002) observed low levels of genetic structure in this species around New Zealand, aside from the populations north of Banks Peninsula, which they inferred was the result of the Southland Current diverging off to the Chatham Islands. In contrast, a genetic discontinuity was not observed for *C. radians* in this region. Instead, extensive haplotype sharing between populations north and south of Banks Peninsula was evident. The discordant genetic structure of these three species suggests that the diverging Southland Current may not explain all the genetic structure observed in this region, particularly as *C. ornata* and *C. radians* both have a high frequency haplotype shared across this region. It may be that while the larvae of

C. ornata and *C. radians* are potentially incapable of long distance dispersal (Anderson, 1962) their larval behaviour or reproductive periods differ in such a way that they are transported in different currents (Hendry and Day, 2005). For instance, gonad indices for *C. ornata* suggest that spawning occurs once a year during late Summer, early Autumn (Dunmore and Schiel, 2000). In contrast, *C. radians* spawns several times throughout the year (Creese and Ballantine, 1983).

The presence of low frequency haplotypes shared across this zone for *Cellana* species may also be remnant of continuous historical distributions (Templeton et al., 1995) from a time when the Banks Peninsula volcano was not joined to the mainland (Stevens et al., 1995). It is possible therefore that the partial isolation of populations north and south of Banks Peninsula reflect this geologically recent barrier to marine dispersal along the east coast, which is persisting due to the offshore direction of the Southland Current that may once have continued straight up the coast to Cape Campbell and beyond (Stevens et al., 1995). This would also explain the low frequency haplotypes occurring in east coast populations of the North Island.

By contrast, Kojima et al. (1997), found a similar pattern of low frequency mitochondrial DNA haplotypes shared between isolated populations of the Japenese snail *Turbo (Batillus) cornutus*. These authors suggested that the low frequency haplotypes were due to recent secondary contact through the Kanmon Strait, which formed about 5000 ya.

C. ornata and *C. radians* both exhibit shallow genetic structure of 0.3% - 1.2% haplotype divergence (Chapter III). However, there is a considerable difference in haplotype frequencies around Cook Strait for these species. *C. ornata* represents an almost complete replacement of haplotypes (Fig 4.5) which is clearly maintained by the lack of substantial migration observed across the barrier (Fig. 4.6a). In contrast, there is considerable haplotype sharing across the barrier in *C. radians.* Two high frequency haplotypes are shared among the North Island and South Island populations of *C. radians*. The almost complete replacement of haplotypes for *C. ornata* was explained by allopatric fragmentation and subsequent radiation within islands (Chapter III). Allopatric fragmentation was also evident for *C. radians* although population expansion was not restricted to within island radiation.

If Cook Strait is not presenting a barrier to dispersal and allopatric fragmentation was not the primary factor isolating *C. radians* populations then changes in sea surface temperature (SST) may explain the observed genetic bottleneck for this species. Species retreats and subsequent range expansion have been reported for many taxa in Europe (Taberlet et al., 1998), the English Channel (Jolly et al. 2005; Provan et al., 2005) and the Northern Atlantic (Wares, 2002). These taxa all showed genetic diversity, and radiations consistent with range expansions. However, *C. radians* did not show a shift in haplotype frequency to the north as might be expected if this species was affected by SST changes during the last glacial maximum (Kirby, 2000).

A recent report on potential larval dispersal from the Te Tapuwae O Rongokako Marine Reserve at Tatapouri Point (Stephens et al., 2004), modelled the pattern of settlement for several intertidal species in varying wind conditions. *Cellana* was one of the genera represented. The report showed that under average, calm and southerly storm conditions with a dispersal period of 2-10 days, larvae released at Pariokonohi Point would be swept northeast for approximately 5km before the majority were swept offshore at Whangara Island with less than 0.01% of the release making it any further north. Greater than 10% of the modelled larvae settled within 1km north or south of the release point. In contrast, during simulated easterly storm conditions the modelled larvae were swept 5km in the opposite direction, towards the south west.

The dispersal model suggests that very short distances are covered by onshore dispersal of all the species. Echinoderm larvae, modelled with a larval period of 4 weeks were also travelling within 5km of the release points in any appreciable numbers. If this model represents an accurate measure of larval dispersal then the stepping stone model of dispersal could be assumed for all these intertidal species and isolation by distance should be evident (Hellberg, 1995). However, *C. ornata* and *C. radians* did not show isolation-by-distance along the east coast, suggesting that dispersal may be more complex than the model implies.

Nielsen and Wakeley's (2003) test for migration and isolation produced low resolution results in the Tatapouri region for both species. This might suggest that a large number of migrants are exchanged between Tatapouri and Cape Kidnappers

populations, as compared to the minimal migration of *C. ornata* across Cook Strait. Alternatively, the low resolve in *C. radians* data may be due to the signature of range expansion producing a pattern indistinguishable from contemporary dispersal between distant populations (Hellberg, 1995).

If *C. radians* larvae are transported in the nearshore system of eddies along the east coast (Fig. 4.1), then southward migration and connectivity between the North and South Island populations may occur. A bottleneck signature may therefore be evident due to the transient nature of the oceanographic anomalies creating ongoing founder events between populations and between islands.

It is clear from the data presented in this chapter that further work on the dispersal potential of *Cellana* limpets and other species is required to more accurately assess the effects of onshore versus nearshore circulation for larval transport. Further sequencing of additional loci would also be advantageous in determining the influence of historical, contemporary or demographic factors on the genetic structure of *C. radians*.

None-the-less, it is evident that phylogeographic structuring around New Zealand matches to some degree, the adjacent nearshore complexity in current systems. Genetic homogeneity occurs along shores with known complexity in the nearshore currents such as the southern regions of the Southland Current and the northern, East Auckland Current. The more complex oceanic regions such as north of Banks Peninsula, East Cape and Cook Strait regions exhibit a more complex signal of genetic diversity, differentiation and haplotype sharing. A similar pattern of low diversity with along-shore flow was found in the Japanese snail, *Turbo (Batillus) cornutus* which maps to two main currents that run northward on different sides of the Honsyu and Kyusyu islands (Kojima et al., 1997). These currents appear to maintain two distinct genetic lineages either side of the islands with genetic homogeneity along each coast line. Like *Cellana*, more complex habitats such as those in the Indo-West Pacific have also been associated with higher genetic differentiation between populations and greater diversity within populations (Lavery et al., 1996).

Chapter V

In Search of Phylogeographic Concordance of New Zealand's Marine Biota

Molecular studies on coastal marine taxa have been carried out in New Zealand for 25 years. Here I review these studies to date and search for concordance among taxa. In previous chapters I have assessed the geographic concordance of genetic discontinuities among closely-related species of *Cellana* around New Zealand. To further investigate phylogeographic processes acting on New Zealand coastal marine biota, I have combined my data with published studies to seek concordance of genetic structure across co-distributed taxa. Concordance of genetic partitions with geographic and biogeographic boundaries was also assessed. Broad genetic similarities across taxa were observed through haplotype mapping, and historical distributions consistent with sea-level changes through the Pliocene and Pleistocene periods were evident. Discordance between the biogeographic provinces and the pattern of genetic differentiation highlight the historical nature of species biogeographic ranges and discontinuities throughout New Zealand. It is clear that baseline data are now available for focused, hypothesis driven studies to further investigate phylogeographic processes acting on the coastal marine communities of New Zealand.

5.1 Introduction

Phylogeographic studies have expanded the field of population genetics by combining geographic distribution and evolutionary processes to make sense of the population genetic structure observed among closely related lineages (Avise, 2004). The field of phylogeography was initially based around the use of the rapidly evolving mitochondrial DNA for intraspecific genealogical studies (Avise et al., 1987) and has expanded rapidly with over 1,500 studies reported by 2000 (Avise, 2000). Many of these studies include phylogeographic concordance between taxa based on the three aspects of 'genealogical concordance' outlined by Avise (1996). These include concordance within a species across multiple unlinked loci, concordance in the geographic position of genetic discontinuities across co-distributed species, and concordance of genetic partitions with geographical boundaries (Avise et al., 1987; Avise, 1998,2004).

In previous chapters, I have used comparative phylogeographic analysis of closely related, co-distributed species with identical larval life histories, to investigate process acting on marine intertidal species around New Zealand. Concordance between *C. ornata* and *C. radians* has shown that geological change around New Zealand has influenced the distribution of genetic variation for each species (Chapter III and IV). In contrast, studies on single species suggest that contemporary oceanic anomalies act as barriers to marine dispersal around the coast of New Zealand (Apte and Gardner, 2002; Star et al., 2003; Waters and Roy, 2004).

New Zealand is located within the Pacific Ocean, spanning 13^{o} of latitude, surrounded by deep ocean and complex coastal currents, straddling the sub-tropical convergence (Heath, 1985). This means that the marine biota is influenced by tropical and sub-tropical waters and is subjected to strong environmental gradients and ephemeral oceanic processes (Heath, 1985). Given these characteristics and the many endemic taxa (Powell, 1979), phylogeographic studies of New Zealand species should be a major contributor to phylogeographic theory.

In order to further investigate the influence of New Zealand's oceanographic and historical processes on intraspecific genetic partitioning, I have reviewed the growing contribution of molecular studies on coastal marine taxa of New Zealand and use some of these studies in a meta-analysis with data from my previous chapters;

first to seek concordance across co-distributed taxa (Avise 2000) and second to explore concordance with geographic and biogeographic boundaries (Avise, 2004) already identified in New Zealand (Powell, 1925; Pawson, 1961; Moore, 1961; Francis, 1996).

There are many illustrative examples of phylogeographic concordance in other parts of the world, both within genera and across diverse co-distributed taxa, in the marine environment. Avise (2004) has been instrumental in advancing phylogeographic concordance, using comparative analyses on a wide array of taxa from terrestrial, marine and freshwater environments. Avise and others (see review by Avise, 2004) have used comparative analyses to examine the influence of marine barriers such as the Amazon basin, the division between the Black Sea and the Caspian Sea, and the Indo-West Pacific boundaries, among others (Avise, 2004).

A comparative study of published data on marine phylogeography in California showed that marine taxa with greater dispersal ability generally had less phylogeographic structure along the Californian coast than taxa with short-lived larvae (Dawson, 2001). However, at the Point Conception transition zone, most of the taxa exhibited a genetic break. Wares et al. (2001) also compared the phylogeographic structure of four taxa in this region to examine whether the ocean currents on the Californian coast were influencing the genetic and species discontinuities observed around Point Conception. These authors found that the three species with pelagic larvae showed a bias toward southward dispersal, following the currents, whereas the one species with crawling larvae showed an excess of northward migration, beyond Point Conception. This is consistent with range expansion that has not been covered by southward dispersal. These three studies highlight the interpretive power of comparative analyses.

5.2 A Review of molecular studies in New Zealand

5.2.1 Published Literature

To date there have been 17 published studies where the intraspecific genetic structure of a New Zealand coastal marine species has been examined (Table 5.1). These studies involve 11 species from 10 genera, undertaken from 1980 to 2005. Of these 17 studies, 9 have used allozyme electrophoresis, 2 used nuclear sequence and 5 used mitochondrial sequences to examine the intraspecific population structure of a single species. A single study compared two closely related species with allozyme markers (Stevens and Hogg, 2004) and only one study used multiple genetic markers within a species, which included a nuclear (ITS) and mitochondrial gene (16S rRNA) (Sponer and Roy, 2002).

A common feature of these coastal studies was the low genetic differentiation between populations of all species (Table 5.1), with six of the studies finding no evidence for genetic structure across broad geographic scales (Smith et al., 1980; Smith et al., 1986; Ovenden et al., 1992; Gardner et al., 1996; Mladenov et al., 1997; Apte and Gardner, 2001). However, clear genetic differentiation between pooled samples of the North and South Island was shown in seven of the studies (Intasuwan et al., 1993; Apte and Gardner, 2002; Perrin, 2002; Sponer and Roy, 2002; Star et al., 2003; Stevens and Hogg, 2004; Waters and Roy, 2004). The north-eastern region of the North Island was also shown to be genetically separate from the rest of New Zealand in four of the studies (Smith, 1988; Smith et al., 1989; Stevens and Hogg, 2004; Waters and Roy, 2004). In addition, local scale genetic structure was observed within Fiordland (Mladenov et al., 1997; Perrin, 2002; Miller et al., 2004), where populations within and between different Sounds were significantly different, and all fiord populations were genetically isolated from coastal populations. These results are consistent with the comparative results from Point Conception (Dawson, 2001) and may therefore suggest that like Point Conception, a barrier to marine dispersal is disrupting the distribution of genetic variation beyond Cook Strait.

In comparison, some commercial marine fish species of New Zealand exhibit genetic homogeneity over broad geographic scales and are not disrupted by Cook Strait. Populations of the orange roughy on the east coast and across Chatham Rise

have been examined in four different studies with allozyme markers, DNA fingerprinting and
mtDNA (Baker et al., 1992; Smolenski et al., 1993; Smith et al., 1996; Smith and Benson, 1997). All four studies report an absence of genetic structure along the east coast of New Zealand (Baker et al., 1992; Smolenski et al., 1993; Smith et al., 1996; Smith and Benson, 1997), but show significant differentiation between New Zealand and the Chatham Rise. In contrast, microsatellite markers for New Zealand snapper *Pagrus auratus*, revealed a clear genetic separation between the northern populations (north of East Cape) and southern populations from the North and South Islands (Bernal-Ramirez et al., 2003).

The different genetic structure observed between the fish species and the coastal invertebrates may be caused by different prevailing currents acting along the coastal environment and the deep continental shelf. Alternatively, coastal organisms are more exposed to geological change (Kauffman and Harries, 1996) and may therefore be genetically structured as a result of sea-level or temperature change. This brief outline of studies cannot resolve the many possible explanations that have been offered for the generation of these genetic patterns without considering the number and location of populations and the genetic markers chosen for each study (Table 5.1). Disparity between sampling regime and marker choice can often result in very different inference of phylogeographic process. For example, Wares (2002) attempted to collate and review the molecular literature of marine phylogeography of the Northwestern Atlantic intertidal community. Unfortunately the disparity in sampling regime between studies meant that only the general findings could be reported, leaving areas of genetic discontinuity and phylogeographic processes unresolved.

Table 5.1. Summary of collection information and molecular results for studies of New Zealand coastal marine taxa.

Taxa	Genetic Marker	Number of loci/ base pairs	Number of Populations	Sample size of populations	Purpose of Study	Results	Reference
Jasus edwardsii	Allozymes	1	3	36-54	Stock assessment	No structure	(Smith et al., 1980)
Jasus edwardsii	mtDNA RFLP	6	2	10	Stock assessment	No structure	(Ovenden et al., 1992)
Crassostrea gigas	Allozymes	9	2	28-52	Stock assessment	No structure	(Smith et al., 1986)
Perna canaliculus	Allozymes	5	6	18-65	Stock assessment	Northeast isolated	(Smith, 1988)
Perna canaliculus	Allozymes	7	10	4-140	Population structure	No structure	(Gardner et al., 1996)
Perna canaliculus	Allozymes	7	35	22-39	Population structure	No structure	(Apte and Gardner, 2001)
Perna canaliculus	mtDNA NADH IV	391bp	22	26	Population structure	North-south split West coast split	(Apte and Gardner, 2002)
Perna canaliculus	RAPD	21 bands	19	20-31	Population structure	North-south split West coast split	(Star et al., 2003)
Paphies subtriangulata	Allozymes	4	13	10-110	Stock assessment	Northeast isolated	(Smith et al., 1989)
Evechinus chloroticus	Allozymes	5	6	18-68	Stock assessment	Fiords structured	(Mladenov et al., 1997)
Evechinus chloroticus	Mircrosatellites	6	8	30-40	Population structure within fiords	Fiords structured North-south split	(Perrin, 2002)
Amphipholis squamata	mtDNA 16S	-	16	4-17	Phylogeography	North-south split	(Sponer and Roy, 2002)
Coscinasterias muricata	mtDNA control	318	17	17-30	Population structure	Genetic cline within sounds	(Perrin, 2002)
Patiriella regularis	mtDNA control	800bp	22	4-7	Phylogeography	North-south split	(Waters and Roy, 2003)
Paracorophium lucasi	Allozymes	10	18	11-35	Phylogeography	North-south split Northeast isolated	(Stevens and Hogg, 2004)
P. excavatum	Allozymes	10	21	5-73	Phylogeography	North-south split Northeast isolated	(Stevens and Hogg, 2004)
Errina novaezelandiae	Allozymes	9	9	7-39	Conservation	Fiords structured	(Miller et al., 2004)

5.2.2 Genetic homogeneity across taxa

Genetic homogeneity across a species range is characteristic of a panmitic population comprising a single gene pool (Hartl and Clark, 1989). This implies that populations are not isolated and gene flow or effective migration is high between them. Several factors can be attributed to the genetic homogeneity of populations. For instance species with teleplanic larvae have the potential to disperse long distances (Scheltema and Williams, 1983), complex larval behaviour may facilitate dispersal in along-shore currents (Forward et al., 2003), or reproductive timing might predict whether larvae are transported onshore or are retained by coastal upwelling events (Hendry and Day, 2005). However, if the sampled populations are not representative of a species range then genetic homogeneity between populations can only serve to show connectivity between the populations sampled and are not necessarily suitable for investigating processes acting on the genetic structure of a species (Wares, 2001).

For instance, five of the 17 New Zealand studies undertaken to date were stock assessment projects (Table 5.1) that focused on the connectivity between two populations or fishery regions rather than the processes involved in genetically structuring populations (Smith et al., 1980; Smith et al., 1986; Smith et al., 1989; Ovenden et al., 1992; Mladenov et al., 1997). These studies used allozymes and predominantly focused on identifying the broad genetic partitions among commercial fisheries species across New Zealand.

The first of these was on the genetic structure of rock lobster *Jasus edwardsii,* (Smith et al., 1980). Of the thirty three allozyme loci examined there was only one polymorphic locus (Est-1). No significant genetic differentiation was observed for this locus across three widely distributed populations (Gisborne, Wellington and Stewart Island). These authors concluded that *Jasus edwardsii* comprised a single stock throughout New Zealand or that selective forces were similar across stocks, at least for that locus. Ovenden et al. (1992) followed up on this work using mtDNA RFLPs to examine the phylogeographic structure of rock lobster throughout Australasia. Again, no genetic sub-division between populations either within New Zealand or across the Tasman Sea was observed. Unfortunately, as for Smith et al. (1980), only east coast populations (Moeraki and Gisborne) were sampled from New Zealand. However, genetic homogeneity of this species is well supported by the

concordance across the seven genetic markers from these combined studies (Table 5.1). The rock lobster does have teleplanic larvae and a strong ability to swim vertically (Ovenden et al., 1992), so genetic homogeneity among widely distributed populations is not impossible. However, the distribution of genetic variation examined across only three east coast populations from a New Zealand wide distribution is unlikely to provide species wide representation.

Similarly, Mladenov et al. (1997) used five polymorphic allozyme loci to investigate the genetic structure of the commercially fished sea urchin *Evechinus chloroticus* across six locations around New Zealand (Leigh, Gisborne, Kaikoura, Dunedin, Stewart Island and Doubtful Sound) (Table 5.1). They found no genetic differentiation between these populations and concluded that currents were not restricting the dispersal of this species, which, like lobsters also possesses teleplanic larvae. They specifically mentioned that the Subtropical Convergence Zone and the southward flowing East Cape Current had no effect on the dispersal of this species, in contrast to their expectations. However, this study did not set out to test hypotheses relating to these two oceanographic features and therefore the sampling regime did not adequately cover areas that may be affected by the hydrographic processes in these regions. Consequently, historical association and selection cannot be ruled out as explanations for the observed genetic homogeneity.

5.2.3 Genetic Discontinuity between the North and South Islands

The patterns of genetic differentiation observed in nine New Zealand marine species (Table 5.1) strongly supports the notion of a phylogeographic break between northern and southern populations. However, it remains unclear where precisely this break occurs. The problem is that while many authors readily infer phylogeographic process, few of the studies have actually sought to address issues of marine phylogeography.

Studies on the green-lipped mussel *Perna canaliculus* provide a good example of how sampling regimes and marker choice can dramatically affect phylogeographic inference. Allozyme electrophoresis on 10 loci from 6 populations of *Perna canaliculus* (Kaipara, Tauranga, Castlepoint, Wellington, Oamaru and Bluff) (Table5.1) showed a genetic discontinuity between the far north populations (Kaipara

and Tauranga) and the remaining populations (Smith, 1988). It was suggested that directional currents and water temperature difference between the north and the south were driving the observed discontinuity. However, when Gardner et al. (1996) examined seven polymorphic allozyme loci (Table 5.1), two of which were also used by Smith (1988), and included populations from the east coast of the North Island and populations from the central north coast of the South Island they found no evidence to support this north-south split. Apte and Gardner (2001) attempted to resolve the disparity presented by the two earlier studies (Smith, 1988; Gardner et al., 1996) with an extensive sampling regime covering 35 populations throughout New Zealand. Seven polymorphic loci, five of which were used in the earlier studies, showed no genetic structure between populations. After extensive investigation these authors concluded that many of the allozyme loci used in earlier studies were probably under selection, which was causing the disparity in genetic structure seen between the studies (Apte and Gardner, 2001). Neutral markers are used for phylogeographic studies in order that genetic drift and migration are the only forces acting to differentiate or homogenise the populations (Avise, 2000). Therefore if the markers used do not exhibit neutral evolution the patterns of genetic differentiation will not reflect phylogeographic process but will instead show local adaptation or purifying selection specific to the species under selection (Nei and Kumar, 2000).

More recently, larger sample sets and a range of molecular approaches have been used to further resolve the population structure of *Perna canaliculus*. The use of mtDNA and RAPD techniques have revealed a significant amount of genetic partitioning in the Cook Strait region (Apte and Gardner, 2002; Star et al., 2003). Both of these studies concluded that the genetic discontinuity around Cook Strait was caused by upwelling events on the east and west coast of the South Island restricting southward dispersal. Both of these studies carried out extensive sampling across New Zealand (Table 5.1) and were able to show that the northern North Island region was not genetically distinct to other regions, as suggested by Smith (1988). However, as with earlier studies on *Perna canaliculus*, the precise location of the genetic discontinuity could not be confirmed without further, more intensive sampling around Cook Strait (see section 5.3.2 for further explanation).

More extensive spatial coverage was achieved by Stevens and Hogg (2004), who analysed 10 allozyme loci for 53 populations (Table 5.1) to determine the phylogeographic structure of two freshwater amphipod species of the genus *Paracorophium* that exhibit marine larval dispersal. Although the biogeographic range of these two species differs slightly, both species exhibited low levels of gene flow among populations. Significant genetic differentiation occurred between populations north and south of East Cape. They concluded that divergent currents and upwelling events around East Cape restricted connectivity between populations in this region (Stevens and Hogg, 2004). They also found that Cook Strait populations were distinct from eastern populations of the North Island, which they suggested was due to allopatric fragmentation. Concordance between these two closely related species and the extensive sampling across the range of each species lend support to the genetic structuring observed, despite the short, single locus tested. However, the explanation of phylogeographic process suggested by these authors would be better supported if additional populations were examined from within and around East Cape.

The studies outlined in this section highlight the importance of focused sampling for phylogeographic inference. More is not necessarily better, particularly if the populations sampled do not coincide with or represent the region identified as being pivotal in structuring the genetic differentiation between populations.

5.3 Phylogeographic Concordance

From the New Zealand literature of molecular studies on coastal marine taxa, only three studies were identified that could be used to examine phylogeographic concordance between co-distributed taxa (Avise 2000). These selected studies (Table 5.2) all sampled a wide range of populations and used mtDNA to investigate the intraspecific pattern of genetic structure with relation to the sea surface currents around New Zealand and the Cook Strait 'barrier' (Apte and Gardner, 2002; Sponer and Roy, 2002; Waters and Roy, 2004). In addition, the limpet species *Cellana ornata* and *C. radians*, studied in this thesis are suitable for comparison with the published literature and have been included in the meta-analysis.

In order to compare the studies through meta-analysis the raw data from each study were obtained from the authors and re-analysed for haplotype frequencies and haplotypes shared between populations in Arlequin v. 2 (Schneider et al., 2000). Data from Waters and Roy (2004) and Sponer and Roy (2002) were also re-analysed for Analysis of Molecular Variance partitions in Arlequin v. 2 (Schneider et al., 2000). The offshore deep water site was removed from the data by Sponer and Roy (2002). See Chapter III for AMOVA methods.

The four studies in table 5.2 highlight several issues that arise from disparity in sampling regime, including the size and location of samples plus the recent and past history of the species studied. All four studies report moderate to strong phylogeographic structure around the Cook Strait region between North Island and South Island populations (Table 5.2). However, there is a large difference in the amount of genetic differentiation observed between the islands for each taxon.

The genetic structure observed for *C. ornata* (Table 5.2) supports a genetic discontinuity between the North and South Island, while *Perna canaliculus, Patiriella regularis* and *C. radians* show moderate structure in this region (Table 5.2). There was no genetic differentiation observed for pooled North Island and South Island populations of *A. squamata* (Table 5.1). However, when the Kaikoura samples were included with the North Island populations then moderate genetic structure was observed (Table 5.2, alternative group). Similarly, the genetic structure between north and south groupings was increased for *C. ornata* and *C. radians* when the samples from French Pass were included with the North Island samples (Table 5.2, alternative).

Table 5.2. Summary of the habitat, life-history and phylogeographic characteristics of New Zealand coastal marine species studied using mtDNA.

Species	habitat	spawn time	larval duration	mtDNA marker	Structure	Alternative	Φst
C. ornata	Mid intertidal	Autunm	2 - 10 dys	cytb	76%	84%	0.83
C. radians	Mid-low intertidal	Multiple	2 – 10 dys	cytb	4.6%	5.71%	0.14
P. regularis	Low intertidal	Spring/Summer	9 - 10 wks	control	6.80%	-	0.08
P.canaliculus	Low intertidal-subtidal	Spring/Summer	3 - 5 wks	NADH IV	16%	-	0.16
A. squamata	variable	Summer/Autumn	brooded	16S	0%	7.52%	0.50

Note: "Structure" represents the percent contribution of variance between a pooled North Island and a pooled South Island sample in molecular variance analyses presented by the corresponding authors (Apte and Gardner, 2002; Sponer and Roy, 2002; Waters and Roy, 2004). *A. squamata* data were re-anlaysed, removing the deep-water population. The "Alternative" variance is from AMOVA results where alternative north-south groupings have been identified in the respective studies as outlined in the text.

The extensive sampling regimes of all these studies throughout the distribution of the species (Table 5.1) allow comparisons of genetic structure between these co-distributed species (Avise, 2000). Unfortunately, concordance across these species regarding the precise location of the genetic discontinuity reported (Table 5.2) and the phylogeographic processes acting to maintain the genetic structure observed is difficult to obtain. However, general patterns can be extracted from the data that can indirectly help in advancing our knowledge of marine phylogeography in New Zealand. For instance life-history correlations (Fig. 5.1) and historical distribution patterns can be obtained from the degree of genetic variation among populations (Fst; Table 5.2) and the distribution of haplotype frequencies (Fig. 5.2).

5.3.1 Life-History

Larval longevity is often used as a measure against which population structure can be measured (Ayre et al., 1997). This measure is used as a simplification of the dispersal potential of a species so that rank correlations between larval dispersal and genetic structure can be calculated. The expectation is that species with teleplanic larvae show low levels of genetic structure across broad geographic scales, while

species with a short larval period exhibit high levels of genetic structure between populations (Ayre 1997).

The larval duration of the species in this study was not significantly correlated with F-statistics for among group structure (Φ_{CT}) or overall population structure (Φ_{ST}) (r = -0.23 and 0.77 respectively, p > 0.05 for both; Fig 5.1). The influence of spawning period on the observed genetic differences is more difficult to ascertain as *C. radians* has multiple releases throughout the year (Creese and Ballantine, 1993). However, the large difference between *C. ornata* and *C.radians*, (Table 5.2) suggests that spawning period may be more relevant than larval duration. The lack of correlation observed, among the five species, with life-history may suggest that contemporary dispersal is not driving the phylogeographic differences observed between the species or that more precise ecological detail is required for each species to adequately test this assumption. *C. ornata* exhibits a striking amount of structure (76%; Table 5.2) in comparison to the other taxa. *A. squamata* is a brooding species which develops to a juvenile stage without a planktonic period (Sponer and Roy, 2002), yet this species showed no genetic differentiation between the North and South Islands, until Kaikoura was pooled with the North Island populations (Table 5.2).

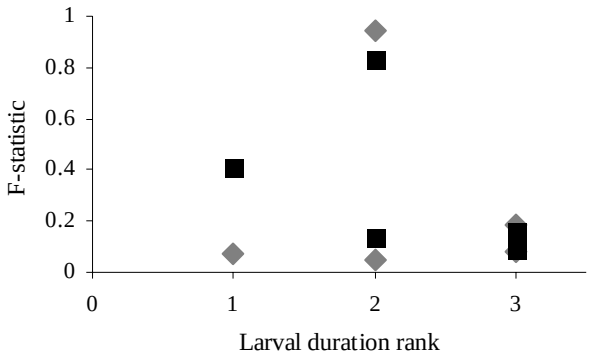

Figure 5.1. Relationships between phylogeographic structure of *A. squamata, C. radians, C. ornata, Patiriella regularis* and *Perna canaliculus* and larval duration rank (1-3). Rank: 1 - brooded young (*A. squamata*); 2 – 1-2 weeks planktonic period (*Cellana* spp.); 3 - > 3 weeks larval duration (*Patiriella regularis* and *Perna canaliculus*). Squares, Φ_{CT}; Diamonds, Φ_{ST} (r = -0.23 and 0.77 respectively, p > 0.05 for both).

The apparent lack of life-history correlation is not unusual, as the expected relationship between life-history and genetic structure is not always observed in comparative studies. For example, the northern hemisphere polychaetes *Neanthes virens* and *Hediste diversicolor* are widely distributed around the cicumpolar region. Planktotrophic larvae of *Neanthes virens* were expected to result in high connectivity between populations (Breton et al., 2003). In contrast, it was expected that populations of the brooding *Hediste diversicolor* would be highly structured. However, moderate to high genetic structure was observed in both species. The demographic history of *N. virens* was shown to disrupt the expected trend (Breton et al., 2003). Likewise, Kyle and Boulding (2000) found that not all of the littorinid species in their comparative study exhibited the expected relationship between genetic structure and life history. Only two of the four species studied exhibited the moderate genetic structuring (brooders) and lack of genetic structure (high-dispersers) expected. These authors suggested that a barrier to dispersal of *Littorina plena* or behavioural constraints were preventing this species from realising its full dispersal potential. Similarly, the nudibranch *Adalaria proxima* showed greater genetic structuring between populations than was expected from the dispersal potential of the species in relation to another nudibranch species, *Goniodoris nodosa*. Again, behavioural constraints were inferred as the cause of this disparity (Todd et al., 1998).

The classification of *A. squamata* as a non-disperser is misleading and may contribute to this species not conforming to characteristic signals of low dispersal species. Sponer and Roy (2002) pointed out that *A. squamata* has been found "rafting" within the stipe of *Macrocystis pyrifera*, possibly facilitating global distribution of the species and probably homogenising the local gene pool. However, the low sample size of each population in this study (n = 4-17) may not be enough to pick up genetic differentiation between populations exhibiting such high diversity observed in this study. Larger sample sizes may be required when genetic diversity, presumably from larger population sizes with an increased mutation rate are observed (Braisher et al., 2004).

Perna canaliculus data indicate high levels of gene flow between populations; however, the data are possibly confounded by two decades of mussel culture, which may have contributed to considerable mixing of populations from different areas of

New Zealand, which is expected to reduce mtDNA diversity (Holland, 2000). Apte and Gardner (2003) did evaluate the introgression of cultured mussel DNA into wild mussel stocks using mitochondrial SSCP, RFLP, and nuclear RAPD markers. They concluded that Stewart Island populations may exhibit genetic introgression from cultured mussels and that wild populations on the west coast of the South Island were genetically differentiated from populations seeded with Kaitaia spat.

5.3.2 Genetic Discontinuity and Historical Distribution

The four phylogeographic studies reviewed here report a genetic discontinuity occurring around the region of Cook Strait. However, the location of the discontinuity is not well resolved, due mostly to the variation in sample locations among the studies (Apte and Gardner, 2002; Sponer and Roy, 2002; Waters and Roy, 2003; chapter III).

Apte and Gardner (2002) reported a genetic break at 42^{o}S (near Kaikoura), coinciding with a region of perceived upwelling. A different inference is obtained through the mapping of haplotype frequencies and the sharing of haplotypes for *Perna canaliculus* (Fig 5.2a). Kaikoura and Westport are the most northern populations to contain the unique South Island haplotype, but there are no samples from within or around the region of perceived upwelling. In addition, the northern South Island populations sampled within the Marlborough Sounds have been seeded from Kaitaia spat for the past 20 – 30 years and may therefore be better considered a representative North Island population. This is also true for four other populations (Houhora, Te Kouma, Golden Bay and Big Glory Bay), all seeded from Kaitaia spat (Apte and Gardner, 2002). It is possible that transplantation of spat has reduced the population differentiation by spreading major haplotypes around the country: four of eight haplotypes occur in all populations. There is one haplotype that is shared among all South Island populations, excluding the northern-most populations. The southern and west coast populations of the South Island carry a low frequency haplotype that is also observed in one population in the north and one population on the north-west coast of the North Island (Fig. 5.2). These low frequency haplotypes with such limited distributions probably contribute all the partitioned variance to the total genetic variation. Therefore, although these authors report a genetic discontinuity coinciding

with a perceived region of upwelling, there is no strong evidence to support the upwelling process as a structuring factor.

Patiriella regularis, C. ornata and *C. radians* have probably not been influenced by anthropogenic alteration of standing stocks. These three species exhibit extensive haplotype sharing (Fig. 5.2), suggesting high connectivity between the populations. However, all populations of *Patiriella regularis* and *C. radians* also contain a high proportion of unique haplotypes (10-90%), while *C. ornata* populations on the northeast of the South Island, Westport and the mid North Island also exhibit unique haplotypes (Fig. 5.2e). *C. radians* has been shown to have experienced a demographic bottleneck (Chapter III and IV). Mismatch distributions of pairwise differences (see Chapter III for explanation of this method) run on *Patiriella regularis* in Arlequin v. 2 (Schneider, 2000) show that the genetic pattern of this species is also consistent with demographic expansion. Further support for a recent expansion is shown by the low divergence between haplotypes (Waters and Roy, 2002). All, but one population (Peppin Bay) support the null hypothesis of population expansion.

In contrast, *A. squamata* shows very little haplotype sharing between populations (Fig. 5.2c), and the sharing occurs over very wide geographic scales. Unlike the other species in this review, haplotype divergence for *A. squamata* is high (Sponer and Roy, 2002), with four divergent lineages containing the 40 haplotypes observed. This pattern would rule out a recent demographic expansion. Sponer and Roy (2002) suggest an ancient divergence which is concordant with other populations of *A. squamata* around the world (Sponer and Roy, 2002). Kaikoura appears to be the point of separation between the North Island and the South Island for *A. squamata* because the two most genetically related lineages dominate separate islands, but both include haplotypes in Kaikoura. The interesting point here is that although the lineages occur around New Zealand and are shared among populations, the haplotypes are not (Fig. 5.2). This lends support to allopatric fragmentation with haplotypes more closely related between populations than within them, in particular the haplotypes between Kaikoura and the North Island. In this instance, further investigation of populations north of Kaikoura on the South Island would help to distinguish between historical association and chance dispersal of rafting adults.

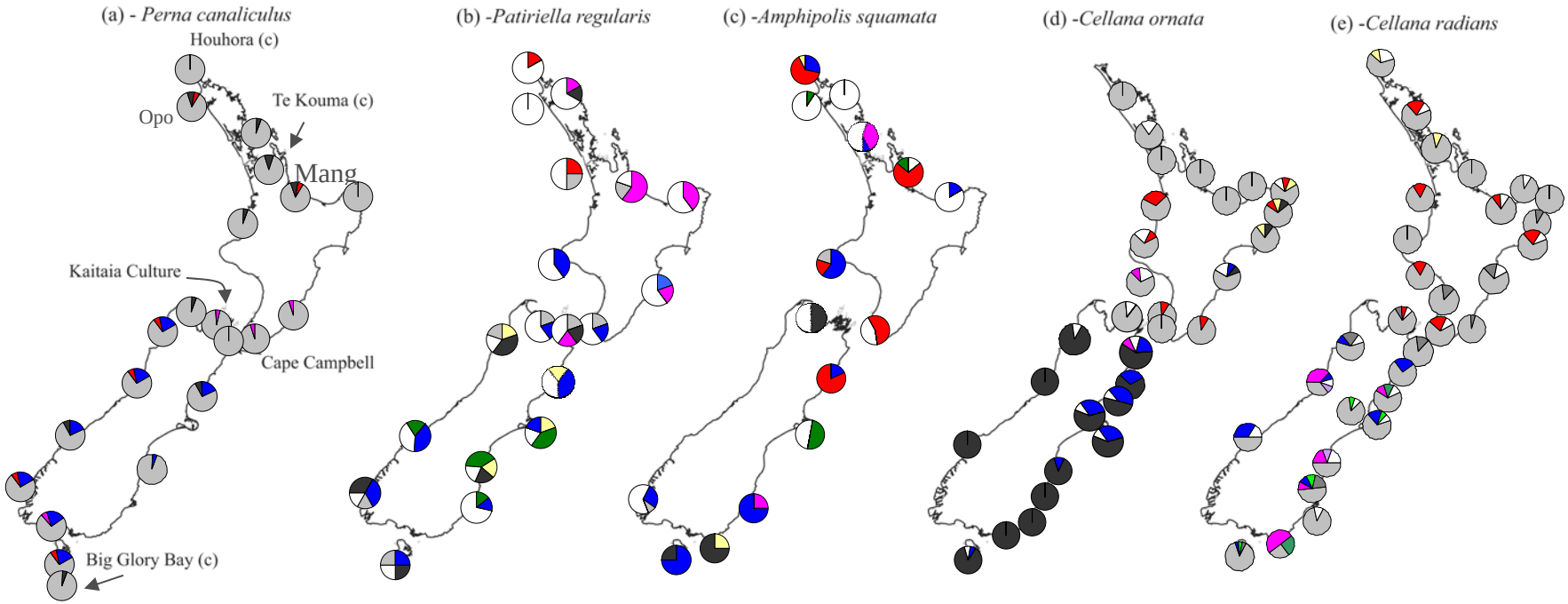

Figure 5.2. Haploytpe distributions of 5 New Zealand coastal marine taxa (a) *Perna canaliculus* (Apte and Gardner, 2002); (b) *Patiriella regularis* (Waters and Roy, 2004); (c) A*mphipholis squamata* (Sponer and Roy, 2002); (d) *Cellana ornata* (Chapter III); (e) *C. radians* (Chapter III). Pie charts represent haplotype frequencies within populations for each taxa. The colours are not consistent across taxa, they represent sharing of haplotypes between corresponding populations. White filled sections represent all the unique haplotypes in a population. Key regions mentioned in text are shown.

Avise's (1987) third aspect of genealogical concordance involves comparisons between genetic pattern and geographic discontinuities. Phylogeographic concordance used in this way can strengthen the inference of a community- wide mechanism for genetic structuring (Avise, 1998; Bermingham and Moritz, 1998; Wares, 2002). Inconsistent phylogeographic patterns suggest that the species have not had a long history of co-association (Taberlet et al., 1998) and that species-specific histories are the more likely structuring factors (Wares et al., 2001).

Haplotype mapping and frequency within populations highlighted some potential patterns of historical distribution and subsequent vicariant disruption across the species. As shown in *C. ornata* a haplotype shared between Cape Campbell and Hawera (Fig. 5.2d) indicates allopatric fragmentation of populations (Templeton, 1995). There is also sharing of South Island haplotypes at very low frequency on the east coast of the North Island (Fig. 5.2d). Together these low frequency haplotypes may indicate that before the separation of Cook Strait *C. ornata* held a continuous distribution along the east coast (Fig 5.3a), which remains detectable at present. The formation of Cook Strait subsequently caused the isolation of the North and South Island populations seen today from which range expansion occurred within each of the islands. It may also indicate that dispersal was in a northerly direction, at least until the opening of the Strait.

Patiriella regularis also shows an association between populations on both the North and South Islands within Cook Strait (Fig. 5.2b). For this species a haplotype dominant within the North Island is also observed in one population of the South Island protruding into Cook Strait (Pepin Island). This pattern is similar to *C. ornata* where an eastern influence is observed, suggesting a relic haplotype in this population (Fig 5.3A). A dominant South Island haplotype is well spread and possibly indicates recurrent northward migration across Cook Strait.

Apte and Gardner (2002) reported two separate mitochondrial lineages from the most common and widespread haplotypes. One lineage constitutes the rare haplotypes occurring predominantly on the South Island but also in several North Island populations (Fig. 5.2a). The second lineage is comprised of haplotypes shared among the North and South Island populations. Without knowing the genetic structure of populations before mussel culturing was initiated, the only informative

haplotypes are those low frequency haplotypes in populations not seeded with Kaitaia spat. This includes only the rare South Island haplotypes that have restricted distributions. In addition, the high number of missing haplotypes in the data set (Fig. 5.2) makes it difficult to identify relic populations.

The genetic pattern of *A. squamata* as discussed earlier is also difficult to discern as the deep phylogenetic divergence of the haplotypes suggests the possibility of cryptic species (Sponer and Roy, 2002), or at least an ancient divergence between taxa that is maintained by restricted gene flow among populations. There is certainly genetic association between Kaikoura and the North Island which supports the theory of a continuous eastern distribution. However, theorising the period at which this would have occurred is beyond the scope of this study. Sponer and Roy (2002) suggested that the pattern of genetic distribution may be due to the sporadic colonisation of New Zealand from South Africa and the North Atlantic, where this species is also widely distributed and some of these overseas lineages are more genetically similar to New Zealand lineages than those within New Zealand.

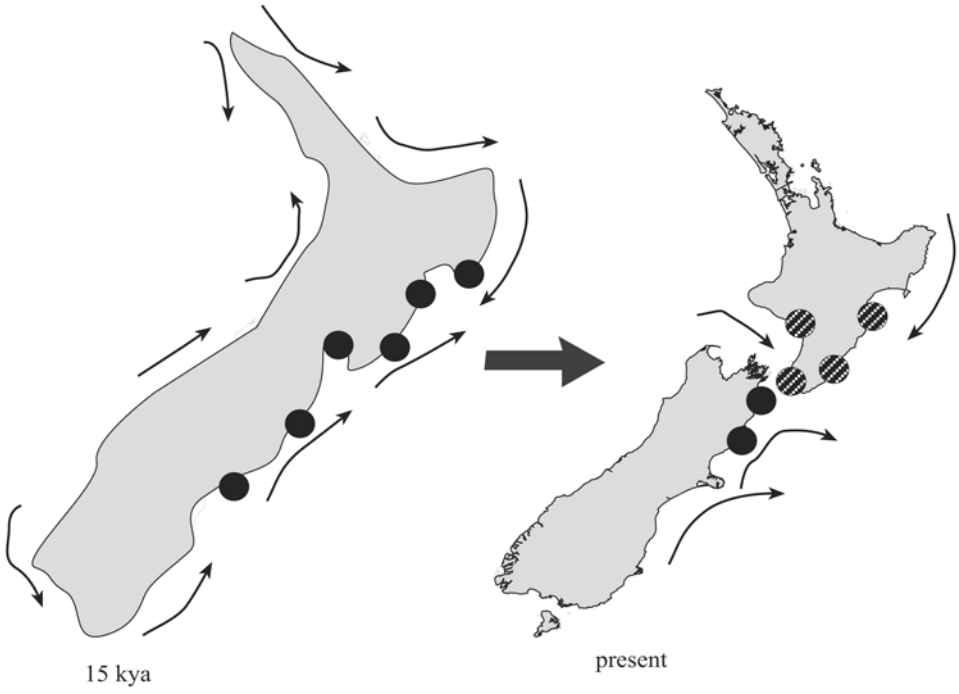

Figure 5.3. Hypothetical representation of the historical association of populations with an eastern distribution distribution. Closed circles represent the historical distribution of haplotypes as suggested in text. Hatched circles represent the fragmentation of the hypothetical distribution with ancestral haplotypes remaining in isolated regions. The maps of New Zealand through time are redrawn from Stephens and Hogg (2004).

5.3.3 Biogeographic Concordance

Having determined the extent of concordance between the taxa and historical association with the geological change in New Zealand, I am now able to look for concordance with independent biogeographic information to assess similarities of speciation and population isolation within New Zealand (Avise, 2000).

Biogeography is a field traditionally based on morphological classification of species and the grouping of these species into range boundaries or provinces. Distinct marine biogeographic provinces of New Zealand have been recognised since the early 1900s when three molluscan provinces (Aupourian, Cookian, Forsterian) were described for mainland New Zealand (Fig. 5.4) (Pawson, 1961 and refs therein). This opened the way for biogeographic studies on other marine taxa. For instance, Pawson (1961) found that the Aupourian province extended down to Cape Egmont for the echinoderms (Fig 5.4b). Four very different provinces were described for the distribution of seaweed species (Moore, 1961), where an intermediate North Island province was added between the Aupourian and Cookian Provinces (Fig. 5.4d). More recently, Francis (1996) suggested that marine fish were restricted to two provinces on the North Island with a third province encompassing the entire South Island (Fig. 5.4c).

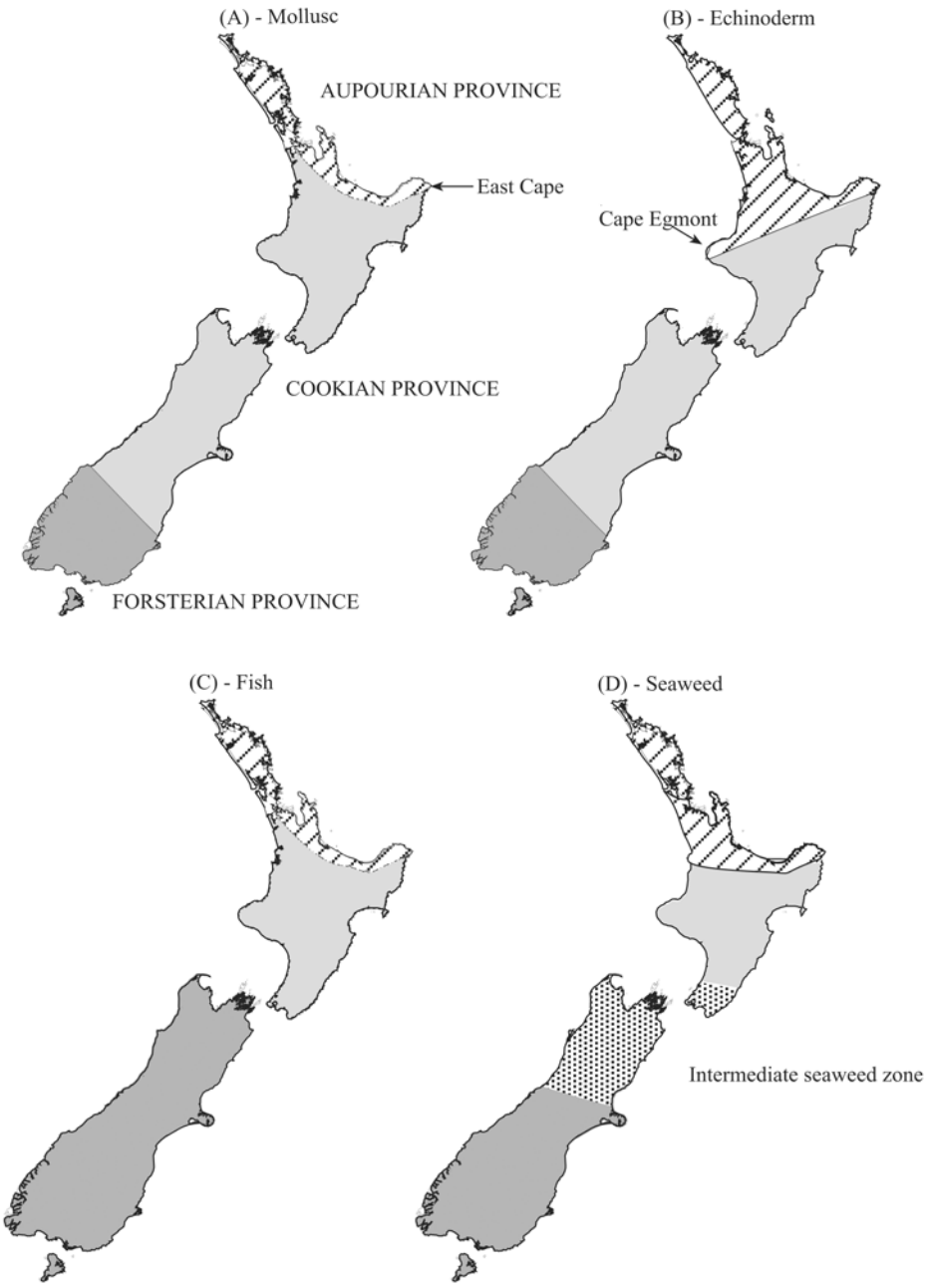

Figure 5.4. Marine Provinces of New Zealand for coastal organisms (A) molluscs (Powell, 1955), (B) echinoderms (Pawson, 1961), (C) marine fish (Francis, 1996) and (D) seaweeds (Moore, 1961). Areas specified in the text are highlighted: East Cape; Cape Egmont; Intermediate seaweed zone; Auporian, Cookian,and Forsterian provinces.

A noticeable pattern across the biogeographic ranges of the taxa presented is the discontinuity at East Cape on the east coast of the North Island (Fig. 5.4). In contrast, boundaries on the west coast overlap across taxa of the North Island. The central Cookian province suggests that Cook Strait is not restricting mollusc and echinoderm taxa from spanning the North and South Islands (Fig. 5.4). In contrast, fish and seaweed taxa both show species discontinuities at the southern region of the North Island (Fig. 5.4). Although, the seaweeds do not appear to be restricted by Cook Strait

Contemporary environmental and ecological factors are often suggested as the cause for biogeographic boundaries. For instance, sea surface temperature clines may exert a selective force (Gaylord and Gaines, 2000), habitat availability may prevent the settling of juveniles (Johanesson et al., 2004) and behavioural traits might result in fish being transported in different currents to the invertebrate and seaweed propagules (Forward et al., 2003). It is well known that the physical characteristics of the coastal water in the northeast nearshore region of the South Island are complex (Heath, 1985) with transient anomalies causing the intrusion of warm water and cold water into the region at monthly and yearly intervals (Uddstrom and Oein, 1999). These anomalies may be responsible for chance dispersal connecting the North and South Islands for some taxa but not for others, due to disparity in reproductive timing.

Biogeographic assignment of marine provinces is based on the morphological taxonomy of species and physical characteristics of the coastal environment. The assignment therefore lacks the power of resolution required to interpret these distributions with respect to ecological or evolutionary processes. The provinces are also surrounded by speculation and debate as to whether the boundaries are an accurate representation of the taxa defined (Dell, 1961). The actual definition of a province varies between authors, suggesting that the lack of resolution may be exacerbated by ambiguity surrounding the classification of a species ‘boundary’ (Dell, 1961).

Population genetics and phylogeography have proved to be powerful tools for assessing contemporary and historical factors that may contribute to the distribution of species and the community composition of marine provinces (Avise, 2000). In addition, genetic studies can discriminate between true species and cryptic species

that cannot be identified by morphology alone. For example, Sponer and Roy (2002) identified at least four highly divergent cryptic brittle star species within the *Amphipholis* genus around New Zealand. In addition, they also reported genetic structuring of *A. squamata* populations concordant with the biogeographic provinces suggested by Pawson (1961) for the echinoderms.

Phylogeography has contributed to the confirmation of biogeographic provinces and faunal discontinuities around the world. For instance, Waters and Roy (2003) found a deep phylogeographic split within the Australian starfish *Coscinasterias muricata*, which was strongly correlated with latitude and the designated marine provinces and suggested an historic association of the discontinuities observed around the southeast coast of Australia.

The molecular studies reviewed in this chapter have contributed to the understanding of biogeographic structure for coastal marine taxa around New Zealand. Sponer and Roy (2002) showed support for Pawson's (1961) echinoderm provinces, but also indicate that the South and North Island are not as connected as Pawson suggests with the identification of cryptic species around the Kaikoura region. *Patiriella regularis* data (Waters and Roy, 2003) also supports the discontinuity of North and South Island echinoderm populations. This disparity highlights the historical association of the east coast whereby species are still present across Cook Strait but their populations may be more isolated than morphology suggests. Likewise, the molluscan genetic studies (Apte and Gardner, 2002; Chapter III) are not concordant with the original marine provinces based on molluscan biogeographic distributions (Powell, 1925). Molecular studies suggest that the Cookian province should be split to reflect the genetic discontinuity around Cook Strait. There is support from most of the molecular studies for a biogeographic discontinuity at or around East Cape as shown by the marine provinces of each taxon (Fig 5.4).

This is not to suggest that the provinces are not accurately represented as they are a collation of contemporary species distributions, but the genetic patterns observed between taxa provide no evidence to suggest that the species distributions are based on contemporary oceanographic factors.

5.4 Summary

A true meta-analysis for the taxa studied in New Zealand is difficult. However, a review of the work that covers the full range of New Zealand coasts shows that there are similar genetic patterns across species along with demographic inconsistencies between taxa. As more comparative studies are carried out, incongruence between taxa is becoming more the rule than the exception (Taberlet et al., 1998). European taxa including birds, mammals, plants and insects, exhibit discordance of phylogeographic pattern (Taberlet et al., 1998). This suggests that each taxon has responded independently to changes during the Quaternary period. Despite this incongruence, colonisation paths were found to be similar among taxa (Taberlet et al., 1998).

In contrast, Wares (2002) observed congruence between marine taxa of the Atlantic boreal shelf region, with a generalised pattern of phylogeographic transition. However, he was unable to resolve the explanation for the generation of the common phylogeographic pattern, probably due to the inconsistencies in sampling regimes between studies. Dawson (2001) also found congruence of phylogeographic pattern among taxa around Point Conception, USA, with some degree of genetic structure common and consistent with phylogeographic hypotheses (Avise, 1987).

It is not unexpected then that the taxa within New Zealand show a varying degree of structure and only weak concordance between taxa. However, the inconsistencies highlight the need for more focused, hypotheses driven sampling of taxa in order to resolve the mechanisms driving the genetic structure of species and communities; particularly with respect to the genetic discontinuity observed around the Cook Strait region. Integration with ecological studies is also a necessary component of comparative analyses in order that demographic disparity such as spawning cues and larval factors can be further addressed.

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Appendices

Appendix I - Laboratory Protocols

A1.1 Lithium Chloride/Chloroform DNA Extraction

DNA extraction followed the protocol of Gemmell and Akiyama (1996), with the addition of an extra lithium chloride step.

Modified from: Gemmell, N. J., Akiyama, S., 1996. A simple and efficient method for the extraction of DNA. Trends Genet. 12 (9), 338-339.

1) Digest: 300 µl isolation buffer
- 50mM TrisHCl
- 50mM EDTA
- 100mM NaCl
- 1% SDS

10µl proteinkinase K (10mg/ml to final concentration of 100mg/µl)
- vortex in 1.5ml tube for 15seconds
- incubate @ 50^{o}C for 2hrs
- store in incubated rocker @ 37^{o}C overnight

2) Wash: 300µl (1 volume) 5M LiCl
- invert 1min

Add 600µl (2 volumes) chloroform to the supernatant
- place on rotating wheel for 30mins
- centrifuge @12000g for 15mins [if interface is cloudy, then repeat this step]

Add 50µl (or 1/10th volume) of 5M LiCl to supernatant
- invert for 1 min
- centrifuge @ 12000g for 10mins

3) Precipitate: Add 600µl (2volumes) room temp 100% ethanol to top layer supernatant.
- invert tube until DNA precipitates
- centrifuge @12000g for 15mins
- carefully pipette off supernatant

4) Wash: Wash the pellet in 600µl (2volumes) 70% ethanol
- centrifuge @12000g for 10mins
- pipette off the supernatant
- dry on the bench for 5mins with lid open

5) Elute: 100µl – 200µl TE8

6) Store: -20^{o}C

7) Confirm: 2µl sample on 1% agarose gel with 0.5x TBE Buffer– electrophorese at 110V for 25-30mins

A1.2. Polymerase Chain Reaction (PCR)

Successful amplification of mitochondrial 12S, 16S and cytochrome b genes was achieved using the following reagents and thermal cycle parameters, as described in chapters II, III and IV.

PCR reaction mix:

25µl Volume	**Final Concentration**	**Stock**	**Supplier**
2.5µl PCR buffer	x1 (50mMKCl,10mM T-HCl)	x10 + MgCl	Roche
		x10 - MgCl	Invitrogen
		x10 - MgCl	Bioline
2.5µl $MgCl_2$	1.5mM	In buffer	Roche
			Invitrogen
			Bioline
2.5µl dNTPs	200µM each	500µl = 10µl each dNTP: 460µlH_2O	Invitrogen
1.25µl each primer	0.5µM	500µl= x10 dilution of 100µM stock	Invitrogen
0.5µl Taq	0.5U	1U/25µl	Roche
0.1µl Taq	0.5U	1U/5µl	Invitrogen
			Bioline
12.5µl PCR H_2O		autoclave, leave in PCR hood	
2µl template DNA	0.4-4ng	1:50 dilutions of extract	

PCR thermal cycle parameters:

Objective	**Temperature (°C)**	**Time (min/sec)**	
Denature	94	2:00	
Denature	94	0:20	repeat x 35
Anneal	47-60	0:20	
Extend	72	0:30	
	72	7:00	

Primer sets used for amplification of mitochondrial genes, 12S, 16S and cytochrome *b*. The product length and the species that were successfully amplified are given:

Name	Sequence	Product/ Success
12S		
12Sma	CTG GGA TTA GAT ACC CTG TTA T	c. 420bp/ all species
12Smb	CAG AGA GTG ACG GGC GAT TTG T	
16S		
16LRN13398	CGC CTG TTT AAC AAA AAC AT	c. 480bp/ all species
16SRHTB	ACG CCG GTT TGA ACT CAG ATC	
Cytochrome *b*		
L14841	CCA TCC AAC ATC TCA GCA TGA TGA AA	c.360bp/ Kosher primers
H15149	CCC CTC AGA ATG ATA TTT GTC CTC A	
CytbP2L	TCC TCA GGG TAA GAC GTA GC	c. 330bp/ *C. ornata*
CytbKdegR	TCT CAG CAT GAT GAA AY TTY	
CytbP2L	TCC TCA GGG TAA GAC GTA GC	c. 330bp/ *C. radians*
CytbKP1R	TCT CAG CAT GAT GAA ACT T	
CytbFHdeg	GTC CTC AGG GTA GAA CAT AAC C	c. 360bp/ *C. flava, C. strigilis*
CytbKdegR	TCT CAG CAT GAT GAA AY TTY	

Appendix II – Phylogenetic Data

Table A2.1. Locality, sample size and identification of New Zealand limpet species collected for phylogenetic study.

Species	Locality	Lat/Long for New Zealand locations	N
Cellana denticulata (Martyn, 1784)	Kaikoura	41.73289S, 174.26950E	5
	Cape Campbell	42.40045S, 173.68992E	5
Cellana flava (Hutton, 1873)	Cape Campbell	41.73289S, 174.26950E	5
	Motunau	43.02539S, 173.07349E	5
	Kaikoura	42.40045S, 173.68992E	5
	Cape Kidnappers	39.63781S, 177.05786E	5
Cellana ornata (Dillwyn, 1817)	Kaikoura	42.40045S, 173.68992E	5
	Woodpecker Bay	42.00904S, 171.37753E	5
	Purangi	36.83885S, 175.81673E	5
	Cape Campbell	41.73289S, 174.26950E	5
	Timaru	35.03343S, 173.91101E	5
	Taupo Bay	34.97401S, 173.69990E	5
Cellana radians (Gmelin, 1791)	Moeraki	42.40045S, 173.68992E	5
	Woodpecker Bay	42.00904S, 171.37753E	5
	Purangi	36.83885S, 175.81673E	5
	Cape Campbell	41.73289S, 174.26950E	5
	Timaru	35.03343S, 173.91101E	5
	Tapotuputu East	34.40805S, 172.97096E	5
Cellana stellifera (Gmelin, 1791)	Taupo Bay	34.97401S, 173.69990E	5
	HaHei	36.83919S, 175.80328E	5
Cellana strigilis rediculum (Reeve, 1854)	Moeraki	45.34575S, 170.84188E	5
	Waipati Beach	46.62407S, 169.35754E	5
	Oamaru	45.08525S, 170.97333E	5
	Timaru	44.38037S, 171.24821E	5
Cellana strigilis chathamensis (Pilsbry, 1891)	Whangatete Inlet , Ch. Isl.	43.47894S, 176.41013W	5
	Te One Creek ,Ch. Isl.	44. 01121S, 176.22985W	5
Cellana strigilis strigilis	Campbell Island	52.50000S, 169.00000E	10
(Hombron and Jacquinot, 1841)	Auckland Island	50.30000S, 166.17000E	10

Table A2.2 . Locality, identification and GenBank accession numbers for 12S/16S published sequences of *Cellana* species used in this study.

Species	Location	Accession #
Cellana radiata capensis (Gmelin, 1791)	Cape Vidal, South Africa	AF058214/ AF058263
Cellana pricei Powell, 1973	O Le Pupu, Upolu, Westerm Samoa	AF058215/ AF058264
Cellana solida (Blainville, 1825)	Oford, Tasmania, Australia	AF058216/ AF058265
Cellana taitensis (Roding, 1798)	Tahiti, French Polynesia	AF058217/ AF058266
Cellana tramoserica (Holten, 1802)	Mollimook, NSW, Australia	AF058218/ AF058267
Cellana testudinaria (Linnaeus, 1758)	Vietnam	AB106431/ AB106480
	Okinawa, Japan	AB106432/ AB106479
Cellana radiata orientalis (Pilsbry, 1891)	Okinawa, Japan	AB106430/ AB106478
Cellana grata (Gould, 1859)	Hong Kong	AB106427/ AB106476
	Mie, Kaino, Japan	AB106428/ AB106475
Cellana nigrolineata (Reeve, 1854)	Mie, Kaino, Japan	AB106429/ AB106477
Cellana toreuma (Reeve, 1855)	Hong Kong	AB106426/ AB106474
Nacella concinna (Strebel, 1908)	Signy Island, Antarctica	AF058219/ AF058268
Scutellastra flexuosa (Quoy & Gaimard, 1834)	Savaii, Western Samoa	AF058183/ AF058231

Note: accession numbers AF058183 – AF058268 (Koufopanou et al., 1999); AB106426 – AB106480 (Nakano and Ozawa, 2004).

Table A2.3. Mitochondrial 12S rDNA sequence alignment, with GenBank accession numbers for New Zealand *Cellana* limpets

```
C. radians (AY621811)          1   AGATACCCTG TTATTGAAAA AAATAAATAT TGATTTACTA TTTGCTTGGG CACTACGAAC
C. flava (AY621809)            1   .......... .......... .......... .......... .......... ..........
C. stellifera (AY621810)       1   .......... .......... .........T .....-.... .......... ..........
C. s. chathamensis (AY621806)  1   .......... .........G .........C .A.AC..... .......... ..........
C. s. denticulata (AY621807)   1   .......... .........G .........C .A.Ac..... .......... ..........
C. s. strigilis (AY627626)     1   .......... .........G .........C .A.AC..... .......... ..........
C. s. redimiculum (AY621808)   1   .......... .........G .........C .A.AC..... .......... ..........
C. s. strigilis (AY627627)     1   .......... .........G .........C .A.AC..... .......... ..........
C. ornata (AY621812)           1   .......... .....AG..G .......... .........- -.C....C... ..........

C. radians (AY621811)         61   ACATGTTTAA AACCCAAAGA ACTTGGCGGC ACTTTAACCC ACTTAGGGGA ACTTGTCCTT
C. flava (AY621809)           61   .......... .......... .......... .......... .......... ..........
C. stellifera (AY621810)      60   .......... .......... .......... .......... .......... ..........
C. s. chathamensis (AY621806) 61   .........T .......... .........T .......... .......... .G........
C. s. denticulata (AY621807)  61   .........T .......... .........T .......... .......... .G........
C. s. strigilis (AY627626)    61   .........T .......... .........T .......... .......... .G........
C. s. redimiculum (AY621808)  61   .........T .......... .........T .......... .......... .G........
C. s. strigilis (AY627627)    61   .........T .......... .........T .......... .......... .G........
C. ornata (AY621812)          59   .......... .......... .........T .......T.. .......... ..C.......

C. radians (AY621811)        121   TAATTCGATA ATCCACGACA ATCTTATTTA TTTTAGAACA CT-TCAGCTT GTATACCGTC
C. flava (AY621809)          121   .......... .......... .......... .......... .......... ..........
C. stellifera (AY621810)     120   .......... .......... .......... .......... .......... ..........
C. s. chathamensis (AY621806)121   .......... .......... .......... .C........ ..C....... ..........
C. s. denticulata (AY621807) 121   .......... .C........ .......... .C........ ..C....... ..........
C. s. strigilis (AY627626)   121   .......... .......... .......... .C........ ..C....... ..........
C. s. redimiculum (AY621808) 121   .......... .......... .......... .C........ ..C....... ..........
C. s. strigilis (AY627627)   121   .......... .......... .......... .C........ ..C....... ..........
C. ornata (AY621812)         119   .......... .C........ .......... C...G..TT. T-CA...... ..........

C. radians (AY621811)        180   GTCACAAGTT AACCTTAAAG AGGATAAAAG TTAACAACAA AGATTAAGAC AAAGGTCTTC
C. flava (AY621809)          180   .......... .......... ..A....... .......T.. .A.......T .........T
C. stellifera (AY621810)     179   .......... .......... ..A....... .......... .........A ...A-.T...
C. s. chathamensis (AY621806)181   ....T..... .......G..A ..A....... .......T.. .....TTA.A .....--T...
C. s. denticulata (AY621807) 181   ....T..... .......G..A ..A....... .......T.. .....TTA.A ...A--T...
C. s. strigilis (AY627626)   181   ....T..... .......G..A ..A....... .......T.. .....TTA.A G..A--T...
C. s. redimiculum (AY621808) 181   ....T..... .......G..A ..A....... .......T.. .....TTA.A G..A--T...
C. s. strigilis (AY627627)   181   ....T..... .......G..A ..A....... .......T.. .....TTA.A G..A--T...
C. ornata (AY621812)         178   ....T..... .........A ..A.A..... .......T.. ....A....T T..A.CT...
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Table A2.3 continue

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C. radians (AY621811)          240  T C T C A G G T C A   G A T C A A G G T G   C A G C T A A T A A   A T A A A G A C A G   A G A T G A G T T A   C A A T T A A C A A
C. flava (AY621809)            240  . . . T . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . T . .
C. stellifera (AY621810)       238  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   G . . . . . . . . .   . . . . . . . . . .   . . G . . . . T . .
C. s. chathamensis (AY621806)  239  . T A . . . . . . .   . . . . . . . . . .   . . . . . . . C . G   . . . . . . . . . .   . . . . . . . C . .   . . . . . . . . . .
C. s. denticulata (AY621807)   239  . T A . . . . . . .   . . . . . . . . . .   . . . . . . . C . G   . . . . . . . . . .   . . . . . . . C . .   . . . . . . . . . .
C. s. strigilis (AY627626)     239  . T A T . . . . . .   . . . . . . . . . .   . . . . . . . C . G   . . . . . . . . . .   . . . . . . . C . .   . . . . . . . T . .
C. s. redimiculum (AY621808)   239  . T A T . . . . . .   . . . . . . . . . .   . . . . . . . C . G   . . . . . . . . . .   . . . . . . . C . .   . . . . . . . T . .
C. s. strigilis (AY627627)     239  . T A T . . . . . .   . . . . . . . . . .   . . . . . . . C . G   . . . . . . . . . .   . . . . . . . C . .   . . . . . . . T . .
C. ornata (AY621812)           238  . . A T . . . . . .   . . . . . . . . . .   . . . . . . . . . .   G . . . . . . - G .   . . . . . G . . . .   . . . . . . . T . .

C. radians (AY621811)          300  A T T T T A C A T G   G A A T T T T C A G   A G A A A A A T G A   A A A A G A A A C A   G G A C T T A A A A   G T A A T T A A T A
C. flava (AY621809)            300  . . . . . . T . . .   . . . . . . . . . T   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. stellifera (AY621810)       298  . . . . . . . . . .   A . . . . . . . . .   . . . . . . . C . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . C .
C. s. chathamensis (AY621806)  299  C . . . . . T . . .   . . . . . . . T . A   . T . . . . . . A .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. s. denticulata (AY621807)   299  C . . . . . T . . .   . . . . . . . T . A   . T . . . . . . A .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . G . .
C. s. strigilis (AY627626)     299  C . . . . . T . . .   . . . . . . . T . A   . T . . . . . . A .   . . . . . . . . T .   . . . . . . . . . .   . . . . . . . . . .
C. s. redimiculum (AY621808)   299  C . . . . . T . . .   . . . . . . . T . A   . T . . . . . . A .   . . . . . . . . T .   . . . . . . . . . .   . . . . . . . . . .
C. s. strigilis (AY627627)     299  C . . . . . T . . .   . . . . . . . T . A   . T . . . . . . A .   . . . . . . . . T .   . . . . . . . . . .   . . . . . . . . . .
C. ornata (AY621812)           297  T . . A . . - . . .   . . . . . C . T . A   . . . . . . T A C G   . . G C A . . . T .   . . . . . . . . . .   . . . . . . T . . G

C. radians (AY621811)          360  A A A A C A A A T G   T T A A T G A A T A   A G G T A A T A A A   G T G T G C A C A A   A T C G C C C G T C   A C T C T C T G A
C. flava (AY621809)            360  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. stellifera (AY621810)       358  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. s. chathamensis (AY621806)  359  . T . . . . . . . A   . . . . . A . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. s. denticulata (AY621807)   359  . T . . . . . . . A   . . . . . A . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. s. strigilis (AY627626)     359  . T . . . . . . . A   . . . . . A . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. s. redimiculum (AY621808)   359  . T . . . . . . . A   . . . . . A . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. s. strigilis (AY627627)     359  . T . . . . . . . A   . . . . . A . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
C. ornata (AY621812)           356  . T . . . . . . C A   C . . . . . . . . .   . . . A . . . . . .   . . . . . T . . . .   . . . . . . . . . .   . . . . . . . . . .
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Table A2.4. Mitochondrial 16S rDNA sequence alignment, with GenBank accession numbers for New Zealand *Cellana* limpets

```
C. radians (AY837762)              1  CAATTAAATA GCCGCGGTAC CCTGACCGTG CAAAGGTAGC ATAATCACTT GCCTCTTAAT
C. ornata (AY837754)               1  T......... .......... .......... .......... .......... ..........
C. s. redimiculum (AY837755)       1  T......... .......... .......... .......... .......... ..........
C. s. striglis (AY837756/57)       1  T......... .......... .......... .......... .......... ..........
C. s. chathamensis (AY837758)      1  T......... .......... .......... .......... .......... ..........
C. denticulata (AY837759)          1  T......... .......... .......... .......... .......... ..........
C. stellifera (AY837760)           1  .......... .......... .......... .......... .......... ..........
C. flava (AY837761)                1  .......... .......... .......... .......... .......... ..........

C. radians (AY837762)             61  TGGTGGCTGG CATGAAAGGT TCAATGAGAA TAAAGCTGTC TCTTTTTTAA TTTATTGAAA
C. ornata (AY837754)              61  ..........      ....G... C.C..... ....A..... ......C... .C........
C. s. redimiculum (AY837755)      61  .......... .......... .T.....A.. ...GA..... .......... ..........
C. s. striglis (AY837756/57)      61  .......... .......... .T.....A.. ...GA..... .......... ..........
C. s. chathamensis (AY837758)     61  .......... .......... .T.....A.. ....A..... ......C... ..........
C. denticulata (AY837759)         61  .......... .......... .T.....A.. ....A..... .......... ..........
C. stellifera (AY837760)          61  .......... .......... .......... ...GA..... .......... ..........
C. flava (AY837761)               61  .......... .......... .......... ....A..... .......... ..........

C. radians (AY837762)            121  CTACCTATTA GGTGAAAAGG CCTAAATAAC TATACAAGAC AATAAGACCC TATCGAGCTT
C. ornata (AY837754)             121  .....C.C.. .......... ....G....T ....T..... .......... ..........
C. s. redimiculum (AY837755)     121  .......... .......... .........CT .......... .......... ..........
C. s. striglis (AY837756/57)     121  .......... .......... .........CT .......... .......... ..........
C. s. chathamensis (AY837758)    121  .......... .......... .........CT .......... .......... ..........
C. denticulata (AY837759)        121  .......... .......... .........TT .......... .......... ..........
C. stellifera (AY837760)         121  .......... .......... .........CT C......... .......... ..........
C. flava (AY837761)              121  .......... .......... .......... C......... .......... ..........

C. radians (AY837762)            181  CAGGCTAAAA TCCATCACAA ATAAAATCAA AAAGAATCTA AGGCCTTTAG TTGGGGCGAC
C. ornata (AY837754)             181  T...T.....  ..T.CT.T.. ......C... ......AT.. CA-.......  .Y.......Y
C. s. redimiculum (AY837755)     181  T...T....C  .....T.A.. .C....A.C. ....G.AA.T  ..A.......  ..........
C. s. striglis (AY837756/57)     181  T...T....C  .....T.A.. .C....A.C. ....G.AA.T  ..A.......  ..........
C. s. chathamensis (AY837758)    181  T...T....C  .....T.A.. .A....A.C. ....G.AA.T  ..A.......  ..........
C. denticulata (AY837759)        181  T...T....C  .....T.A.. .A.G..A.C. ....G.AGCT  ..A.......  ..........
C. stellifera (AY837760)         181  .....C.... CT..CT.... ...G...... ..........T  ..........  ..........
C. flava (AY837761)              181  T....CG...  .T........ .......... ..........T  ..........  ..........

C. radians (AY837762)            241  TAAGGAACAA ACAAAACTTC CTAAAACAAA AACA-CCAAC AAGTTACGAT CCGAAAAATA
C. ornata (AY837754)             240  .......... .......... ......T..T ..T....C.. ....C.T..C ...G......
C. s. redimiculum (AY837755)     241  .......... .......... ......T... .......... ......T... .........A.
C. s. striglis (AY837756/57)     241  .......... .......... ......T... .......... ......T... .........A.
C. s. chathamensis (AY837758)    241  .......... .......... ......T... G......... ......T... .........A.
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Table A2.4 continue

```
C. denticulata (AY837759)  241  . . . . . T . . . . . T . . C . . . . . A -
C. stellifera (AY837760)   241  . . . . . T . . . . . T . . . . . G . . A -
C. flava (AY837761)        241  . . . . . G . . . . . . . . . . . . . . A .

C. radians (AY837762)      300  T T C G A C A A A A  G A A A A A - G C T  A C C G T A G G G A  T A A C A G C G T A  A T T T T C T T T G  T G A G C T C T T A
C. ornata (AY837754)       299  C C . . . T . . . .  . . . . . A . . .  . . . . . A . . .  . . . . . .  . . . . . .  . . . . . .
C. s. redimiculum (AY837755) 300  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .
C. s. striglis (AY837756/57) 300  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .
C. s. chathamensis (AY837758) 300  . . T . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .
C. denticulata (AY837759)  299  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .
C. stellifera (AY837760)   299  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .
C. flava (AY837761)        300  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .

C. radians (AY837762)      359  T T A A A A A G A A  A G T T T G C G A C  C T C G A T G T T G  G A T T A A G G T A  T C C T T A A G A C  G C A G C A G T C
C. ornata (AY837754)       359  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . A . . .  . . . . . .
C. s. redimiculum (AY837755) 359  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . C . . .  . . . . . .
C. s. striglis (AY837756/57) 359  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . C . . .  . . . . . .
C. s. chathamensis (AY837758) 359  . . . . . .  . . . . . .  . . . . . .  . . . . . .  C . . C . . .  . . . . . .
C. denticulata (AY837759)  358  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . C . . .  . . . . . .
C. stellifera (AY837760)   358  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .
C. flava (AY837761)        359  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .  . . . . . .
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Table A2.5. Pairwise disparity index (above diagonal) and probability (below diagonal) for the mitochondrial 12S rDNA sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *C. radians*		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.06	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.08**	**0.00**	**0.00**	**0.44**
2 *C. pricei*	**1.00**		**0.00**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.07**	**0.00**	**0.00**	0.50
3 *C. taitensis*	**1.00**	**1.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.03**	**0.00**	**0.00**	0.52
4 *C. capensis*	**1.00**	**0.30**	**1.00**		**0.00**	**0.01**	**0.01**	**0.03**	**0.09**	**0.09**	**0.09**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**	**0.05**	**0.05**	0.54
5 *C. tramoserica*	**1.00**	**1.00**	**1.00**	**1.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.00**	0.71
6 *C. solida*	**1.00**	**1.00**	**1.00**	**0.31**	**1.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.05**	**0.00**	**0.00**	0.78
7 *C. denticulata*	**1.00**	**1.00**	**1.00**	**0.36**	**1.00**	**1.00**		**0.00**	0.03	0.03	**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.11**	**0.00**	**0.00**	**0.50**
8 *C. s. chathamensis*	**1.00**	**1.00**	**1.00**	**0.27**	**1.00**	**1.00**	**1.00**		**0.01**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.14**	**0.00**	**0.00**	0.57
9 *C. s. strigilis*	**1.00**	**1.00**	**1.00**	**0.16**	**1.00**	**1.00**	0.01	**0.11**		0.00	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.10**	0.21	**0.00**	**0.00**	0.74
10 *C. s. redimiculm*	**1.00**	**1.00**	**1.00**	**0.15**	**1.00**	**1.00**	0.01	**0.12**	0.00		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.10**	0.21	**0.00**	**0.00**	0.74
11 *C. flava*	0.01	**0.19**	**0.25**	**0.14**	**1.00**	**1.00**	**0.25**	**0.34**	**1.00**	**1.00**		**0.02**	**0.00**	**0.08**	**0.05**	**0.04**	**0.06**	**0.17**	**0.19**	**0.11**	**0.11**	1.00
12 *C. stellifera*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.18**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.06**	**0.00**	**0.00**	0.62
13 *C. ornata*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.04**	**0.13**	**0.00**	**0.00**	**0.46**
14 *N. concinna*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.22**	**1.00**	**1.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.31**
15 *C. grata*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.16**	**1.00**	**1.00**	**1.00**		**0.00**	**0.00**	**0.00**	**0.08**	**0.00**	**0.00**	**0.38**
16 *C. grata (CH)*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.20**	**1.00**	**1.00**	**1.00**	**1.00**		**0.00**	**0.00**	**0.04**	**0.00**	**0.00**	**0.47**
17 *C. nigrolineata*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.14**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**		**0.00**	**0.08**	**0.00**	**0.00**	**0.39**
18 *C. toreuma*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.15**	**0.14**	0.03	**0.31**	**0.23**	**1.00**	**1.00**	**1.00**	**1.00**		**0.00**	**0.00**	**0.00**	0.52
19 *C. orientalis*	**0.16**	**0.17**	**0.26**	**0.29**	**0.35**	**0.18**	**0.11**	**0.08**	0.04	0.04	**0.05**	**0.19**	**0.12**	**1.00**	**0.17**	**0.24**	**0.12**	**1.00**		**0.09**	**0.09**	0.87
20 *C. testundinaria*	**1.00**	**1.00**	**1.00**	**0.20**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.13**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.14**		0.00	**0.40**
21 *C. testundinaria (V)*	**1.00**	**1.00**	**1.00**	**0.21**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.12**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.13**	0.00		**0.40**
22 *S. flexuosa*	**0.05**	**0.05**	0.04	0.02	0.01	0.01	**0.05**	0.04	0.01	0.03	0.00	0.03	0.04	**0.09**	**0.08**	**0.06**	**0.08**	0.04	0.01	**0.06**	**0.07**	

Note: significant results are indicated in bold text.

Table A2.6. Pairwise disparity index (above diagonal) and probability (below diagonal) for the mitochondrial 16S rDNA sequences.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *C. radians*		**0.00**	**0.01**	**0.03**	**0.00**	**0.05**	**0.04**	**0.01**	**0.04**	**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.00**	**0.06**	**0.04**	**0.10**	**0.10**	0.62
2 *C. pricei*	**1.00**		**0.00**	**0.07**	**0.00**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.08**	**0.00**	**0.03**	**0.14**	**0.05**	**0.00**	**0.00**	**0.25**	**0.25**	**0.36**
3 *C. taitensis*	**0.32**	**1.00**		0.09	**0.01**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.01**	**0.12**	**0.00**	**0.05**	0.17	0.08	**0.00**	**0.00**	**0.30**	**0.30**	**0.32**
4 *C. capensis*	**0.24**	**0.07**	0.04		**0.00**	**0.00**	**0.06**	**0.04**	**0.07**	**0.07**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**	**0.03**	**0.02**	**0.02**	**0.31**
5 *C. tramoserica*	**1.00**	**1.00**	**0.38**	**1.00**		**0.04**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**	**0.00**	**0.00**	**0.00**	**0.10**	**0.10**	**0.38**
6 *C. solida*	**0.16**	**0.32**	**0.37**	**1.00**	**0.09**		**0.01**	**0.00**	**0.01**	**0.01**	**0.02**	**0.01**	**0.04**	**0.00**	**0.00**	**0.06**	**0.01**	**0.00**	**0.00**	0.13	0.13	**0.18**
7 *C. denticulata*	**0.15**	**1.00**	**1.00**	**0.14**	**1.00**	**0.32**		**0.00**	**0.00**	**0.00**	**0.03**	**0.05**	**0.11**	**0.00**	**0.04**	0.16	**0.07**	**0.00**	**0.00**	0.30	0.30	**0.31**
8 *C. s. chathamensis*	**0.35**	**1.00**	**1.00**	**0.18**	**1.00**	**1.00**	**1.00**		**0.00**	**0.00**	**0.00**	**0.01**	**0.07**	**0.00**	**0.02**	0.12	**0.03**	**0.00**	**0.00**	0.24	0.24	**0.34**
9 *C. s. strigilis*	**0.12**	**1.00**	**1.00**	**0.11**	**1.00**	**0.38**	**1.00**	**1.00**		**0.00**	**0.03**	**0.05**	**0.11**	**0.00**	**0.05**	0.17	**0.08**	**0.00**	**0.00**	0.30	0.30	**0.31**
10 *C. s. redimiculm*	**0.12**	**1.00**	**1.00**	**0.11**	**1.00**	**0.38**	**1.00**	**1.00**	**0.00**		**0.03**	**0.05**	**0.11**	**0.00**	**0.05**	0.17	**0.08**	**0.00**	**0.00**	0.30	0.30	**0.31**
11 *C. flava*	**1.00**	**1.00**	**0.40**	**1.00**	**1.00**	**0.30**	**0.22**	**1.00**	**0.17**	**0.21**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.04**	**0.02**	**0.06**	**0.06**	0.58
12 *C. stellifera*	**1.00**	**1.00**	**0.34**	**1.00**	**1.00**	**0.34**	**0.12**	**0.28**	**0.08**	**0.09**	**1.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.04**	**0.03**	**0.04**	**0.04**	0.55
13 *C. ornata*	**1.00**	**0.07**	**0.04**	**1.00**	**1.00**	**0.18**	**0.05**	**0.12**	**0.07**	**0.05**	**1.00**	**1.00**		**0.06**	**0.00**	**0.00**	**0.00**	**0.10**	**0.08**	**0.00**	**0.00**	0.61
14 *N. concinna*	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.22**		**0.00**	**0.09**	**0.01**	**0.00**	**0.00**	**0.21**	**0.21**	**0.13**
15 *C. grata*	**1.00**	**0.18**	**0.07**	**1.00**	**1.00**	**1.00**	**0.15**	**0.30**	**0.14**	**0.12**	**1.00**	**1.00**	**1.00**	**1.00**		**0.02**	**0.00**	**0.04**	**0.02**	**0.04**	**0.04**	0.46
16 *C. grata (CH)*	**0.33**	**0.01**	0.00	**1.00**	**0.23**	**0.10**	0.01	0.04	0.01	0.01	**1.00**	**1.00**	**1.00**	**0.16**	**0.05**		**0.00**	0.15	0.13	**0.00**	**0.00**	0.65
17 *C. nigrolineata*	**1.00**	**0.12**	0.04	**1.00**	**1.00**	**0.35**	**0.08**	**0.20**	**0.07**	**0.05**	**1.00**	**1.00**	**1.00**	**0.35**	**1.00**	**1.00**		**0.07**	**0.04**	**0.02**	**0.02**	0.53
18 *C. toreuma*	**0.15**	**1.00**	**1.00**	**0.27**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.21**	**0.21**	**0.05**	**1.00**	**0.16**	0.01	**0.06**		**0.00**	0.25	0.25	**0.11**
19 *C. orientalis*	**0.23**	**1.00**	**1.00**	**0.13**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**0.30**	**0.24**	**0.08**	**1.00**	**0.21**	0.02	**0.18**	**1.00**		0.20	0.20	**0.14**
20 *C. testundinaria*	**0.06**	**0.01**	**0.00**	**0.28**	**0.04**	0.03	0.00	0.01	0.00	0.00	**0.17**	**0.23**	**1.00**	**0.05**	**0.18**	**1.00**	**0.24**	0.01	0.01		0.00	0.71
21 *C. testundinaria (V)*	**0.06**	**0.00**	**0.00**	**0.28**	**0.05**	0.02	0.00	0.00	0.00	0.00	**0.15**	**0.24**	**1.00**	**0.05**	**0.17**	**1.00**	**0.28**	0.00	0.02	0.00		0.71
22 *S. flexuosa*	0.02	**0.06**	**0.08**	**0.07**	**0.06**	**0.14**	**0.06**	**0.05**	**0.08**	**0.07**	0.03	0.03	0.02	**0.18**	0.03	0.00	0.03	**0.21**	**0.18**	0.01	0.01	

Note: significant results are indicated in bold text

Appendix III – Phylogeography Data

A3.1. Population site information

The site information and abiotic variables for all the populations sampled in chapter III and chapter IV are set out below.

Table A3.1.1. Locations and identification codes of the sampled populations.

Sample Locations			
South Island	population ID	Longitude	Latitude
Cape Campbell	CC	41.73289S	174.26950E
Diamond Harbour	DH	43.61379S	172.74467E
Dunedin	D	45.80296S	170.74530E
French Pass	FP	40.91915S	173.83356E
Jackson Head	JH	43.96236S	168.61184E
Kaikoura	K	42.40045S	173.68992E
Little Pigeon Bay	LPB	43.59592S	172.81657E
Moeraki	M	45.34575S	170.84188E
Motonau	Mot	43.02539S	173.07349E
Oamaru	Om	45.08525S	170.97333E
Stewart Island	SI	46.89820S	168.12440E
Timaru	Tim	44.38037S	171.24821E
Waipati Beach	WP	46.62407S	169.35754E
West Port	We	41.74223S	171.48534E
Woodpecker Bay	WPB	42.00904S	171.37753E
North Island			
Cape Kidnappers	CK	39.63781S	177.05786E
Castlepoint	CP	40.90632S	176.21273E
East Cape	EC	37.68100S	178.54915E
Hawera	Haw	39.60054S	174.22002E
Island Bay, Wellington	IB	41.34403S	174.68025E
Kopongatahi Point	CR	37.57345S	177.98761E
Koutuni Point	Tok	38.10948S	178.34483E
New Plymouth	NP	39.05842S	174.02068E
North Harbour, Auckland	NH	36.82263S	174.78901E
Matheson Bay	MB	36.32395S	174.74948E
Ohope	Ohop	37.94928S	177.01815E
Pukerua Bay	Kap	41.01842S	174.88604E
Purangi	P	36.81180S	175.69679E
Raglan	Rag	37.80255S	174.83049E
Tatapouri	Tat	38.10948S	178.34483E
Tapotaputu East	TTP	34.40805S	172.97096E
Taupo Bay	TB	37.97401S	173.69990E

Table A3.1.2. Mean annual sea surface temperature (Diagonal), difference in SST (below) and distance in kilometeres (above) between populations.

	CC	CK	CP	CR	D	DH	EC	FP	Haw	IB	JH	K	Kap	LPB	M	MB	Mot	NH	NP	Oh	Om	P	Rag	SI	Tat	TB	Tim	Tok	TTP	We	WP	WPB
CC	**13**	304	810	206	574	273	567	100	214	26	458	127	77	1147	558	274	199	1027	325	717	536	1174	414	827	454	1141	448	516	1141	496	726	546
CK	4	**17**	510	101	577	577	268	373	517	332	1054	431	362	848	862	578	503	728	620	605	840	721	737	1131	343	1005	752	411	1005	713	706	763
CP	2	2	**15**	409	1270	1416	55	681	840	640	1362	937	685	525	1701	1417	1342	405	943	93	1679	298	1060	1970	187	682	1591	106	682	1021	1399	1071
CR	5	1	3	**18**	932	479	354	272	416	231	918	333	261	480	764	934	405	814	519	504	742	707	636	1033	242	1161	654	310	1161	612	1071	662
D	1	5	3	6	**12**	338	1125	673	413	300	682	447	430	337	31	1683	413	1475	1037	1544	52	1494	1053	242	1628	1715	121	1911	1715	1023	129	973
DH	0	4	2	5	1	**13**	840	372	487	299	1019	146	350	1	369	1420	75	1300	598	990	286	1193	687	586	727	1414	217	789	1414	670	467	719
EC	5	1	3	0	6	5	**18**	626	785	585	1307	694	630	841	1125	580	766	460	888	150	1103	353	1005	1394	132	737	1015	51	737	966	1254	1016
FP	2	2	0	3	3	2	3	**15**	157	62	358	226	92	373	657	850	298	970	244	776	635	1273	355	935	514	828	547	582	828	340	825	390
Haw	2	2	0	3	3	2	3	0	**15**	237	692	341	155	924	1405	488	413	1044	103	935	1436	1241	220	1181	653	693	1495	734	693	351	542	401
IB	1	3	1	4	2	1	4	1	1	**14**	743	153	82	1161	584	300	225	1041	288	735	562	1314	405	853	473	930	153	541	930	402	429	452
JH	2	2	0	3	3	2	3	0	0	1	**15**	585	725	1018	657	1531	713	1651	728	1457	734	1632	830	489	1175	1300	802	1256	1300	341	553	291
K	1	3	1	4	2	1	4	1	1	0	1	**14**	204	147	431	1274	72	1154	452	844	409	1047	541	700	581	1268	321	643	1268	512	599	623
Kap	2	2	0	3	3	2	3	0	0	1	0	1	**15**	351	635	1027	276	1147	258	780	613	1396	375	904	498	848	525	579	848	384	559	434
LPB	1	3	1	4	2	1	4	1	1	0	1	0	1	**14**	368	1421	76	1301	599	991	285	1194	688	585	728	1415	216	790	1415	669	466	720
M	0	4	2	5	1	0	5	2	2	1	2	1	2	1	**13**	1705	444	1585	1441	1275	83	1478	1543	383	1012	2013	152	1074	2013	1054	160	1004
MB	5	1	3	0	6	5	0	3	3	4	3	4	3	4	5	**18**	1346	120	821	430	1683	227	704	1859	712	231	1595	631	231	1190	1812	1240
Mot	0	4	2	5	1	0	5	2	2	1	2	1	2	1	0	5	**16**	1226	524	916	361	1119	613	661	653	1340	292	715	1445	584	542	634
NH	5	1	3	0	6	5	0	3	3	4	3	4	3	4	5	0	5	**18**	941	310	1563	107	824	1854	592	491	1475	511	596	1310	1604	1360
NP	3	1	1	2	4	3	2	1	1	2	1	2	1	2	3	2	3	2	**16**	1038	1472	1138	117	1217	756	590	1531	837	485	387	1166	437
Oh	5	1	3	0	6	5	0	3	3	4	3	4	3	4	5	0	5	0	2	**18**	1253	203	1155	1544	282	587	1165	201	692	1116	1673	1166
Om	0	4	2	5	1	0	5	2	2	1	2	1	2	2	0	5	0	5	3	5	**13**	1456	1574	300	990	2044	68	1052	2149	1085	187	1035
P	5	1	3	0	6	5	0	3	3	4	3	4	3	4	5	0	5	0	2	0	5	**18**	1021	1632	485	384	1368	404	489	1747	1646	1670
Rag	4	0	2	1	5	4	1	2	2	3	2	3	2	1	4	1	4	1	1	1	4	1	**17**	1319	873	473	1633	954	1738	486	1182	536
SI	1	5	3	6	0	1	6	3	3	2	3	2	3	4	1	6	1	6	4	6	1	6	5	**12**	1281	1789	368	1343	473	830	113	780
Tat	4	0	2	1	5	4	1	2	2	3	2	3	2	3	4	1	4	1	1	1	4	1	0	5	**17**	869	902	88	1007	834	1757	884
TB	5	1	3	0	6	5	0	3	3	4	3	4	3	4	5	0	5	0	2	0	5	0	1	6	1	**18**	2103	789	105	959	1586	1009
Tim	0	4	2	5	1	1	5	2	2	1	2	1	2	1	0	5	0	5	3	5	0	5	4	1	4	5	**13**	964	2208	1144	250	1094
Tok	4	0	2	1	5	4	1	2	2	3	2	3	2	4	4	1	4	1	1	1	4	1	0	5	0	1	4	**17**	894	915	1830	965
TTP	5	1	3	0	6	5	0	3	3	4	3	4	3	4	5	0	5	0	2	0	5	0	1	6	1	0	5	1	**18**	1064	1691	1114
We	2	2	0	3	3	2	3	0	0	1	0	1	0	2	2	3	2	3	1	3	2	3	2	3	2	3	2	2	3	**15**	898	50
WP	2	6	4	7	4	2	7	4	4	3	4	3	4	4	2	7	4	7	5	7	2	7	6	1	6	7	2	6	7	4	**11**	848
WPB	2	2	4	3	3	2	3	0	0	1	0	1	0	1	2	3	2	3	0	3	2	3	2	3	2	3	2	2	3	0	4	**15**

A3.2 Phylogeographic data

The following genetic data describing the cytochrome *b* gene is presented separately for each species

- Haplotype alignment
- Diversity indices
- Haplotype frequency table for each population
- Population differentiation distances (d_{XY} (above diagonal); d_A (below diagonal); d_X (diagonal).

Cellana ornata

Table A3.2.1 Haplotype alignment for 21 C. ornata cytochrome *b* haplotypes.

01.O2K	1	T T C T C A G C A T	G A T G A A A T T T	C G G A T C T C T T	T T A G G G T T A T	G T T T A A T T A T
02.O73LPB	1	. . . .	. . . .	. . . .	. . . .	. . . .
03.O93DH	1	. . . .	. . . .	. . . .	. . . .	. . . .
04.O16CC	1	. . . .	. . . .	. . . .	. . . .	. . . .
05.O18CC	1	. . . .	. . . .	. . . .	. . . .	. . . .
06.O17CC	1	. . . .	. . . .	. . . .	. . . .	. . . .
07.O11P	1	. . . .	. . . .	. . . .	. . . .	. . . .
08.O221NP	1	. . . .	. . . .	. . . .	. . . .	. . . .
09.O222NP	1	. . . .	. . . .	. . . .	. . . .	. . . .
010.O116CP	1	. . . .	. . . .	. . . .	. . . .	 C
011.O123MB	1	. . . .	. . . .	. . . .	. . . .	. . . .
012.O212TOK	1	. . . .	. . . .	. . . .	. . . .	 C
013.O105MOT	1	. . . .	. . . .	. . . .	. . . .	. . C . . . .
014.O150SI	1	. . . .	. . . .	. . . .	. . . . . C	. . . .
015.O138FP	1	. . . .	. . . .	. . . .	. . . .	. . . .
016.O174EC	1	. . . .	. . . .	. . . .	. . . .	 C
017.O166WE	1	. . . .	. . . .	. . . .	. . . .	. . . .
018.O253HAW	1	. . . .	. . . .	. . . .	. . . .	. . . .
019.O255HAW	1	. . . .	. . . .	. . . .	. . . .	. . . .
020.O235CK	1	. . . .	. . . .	. . . .	. . . .	. . . .
021.O233CK	1	. . . .	. . . .	. . . .	. . . .	. . . .
01.O2K	51	A C A A A T C T T A	A C A G G A C T A T	T T T T A T C T A T	A C A C T A C A C C	G C T A A T A T T G
02.O73LPB	51	. . . .	. . . .	. . . .	. . . .	. . . .
03.O93DH	51	. . . .	. . . .	. . . .	. . . .	. . . .
04.O16CC	51	. . . .	. . . .	. . . .	. . . .	. . . .
05.O18CC	51	. . . .	. . . .	. . . .	. . . .	. . . .
06.O17CC	51	. . . .	. . . .	. . . .	. . . .	. . . .
07.O11P	51	. . . .	. . . .	. . . .	. . . .	. . . .
08.O221NP	51	. . . .	. . . .	. . . .	. . . .	. . . .
09.O222NP	51	. . . .	. . . .	. . . .	. . . .	. . . .
010.O116CP	51	. . . .	. . . .	. . . .	. . . .	. . . .
011.O123MB	51	. . . .	. . . .	. . . .	. . . .	. . . .
012.O212TOK	51	. . . .	. . . .	. . . .	. . . .	. . . .
013.O105MOT	51	. . . .	. . . .	. . . .	. . . .	. . . .
014.O150SI	51	. . . .	. . . .	. . . .	. . . .	. . . .
015.O138FP	51	. . . .	. . . .	. . . .	. . . .	. . . .
016.O174EC	51	. . . .	. . . .	. . . .	. . . .	. . . .
017.O166WE	51	. . . .	. . . .	. . . .	. . . .	. . . .
018.O253HAW	51	. . . .	. . . . . . T . . .	. . . .	. . . .	. . . .
019.O255HAW	51	. . . .	. . . .	. . . .	. . . .	. . . .
020.O235CK	51	. . . .	. . . .	. . . .	. . . .	. . . .
021.O233CK	51	. . . .	. . . .	. . . .	. . . .	. . . .
01.O2K	101	A C A C A G C A T T	C T C A T C C G T C	G C C C A T A T C A	C A C G A G A T G T	A A A T T A T G G A
02.O73LPB	101	. . . .	. . . .	. . . .	. . . .	. . . .
03.O93DH	101	. . . .	. . . . . . T . . .	. . . .	. . . .	. . . .
04.O16CC	101	. . . .	. . . .	. . . .	. . . .	. . . .
05.O18CC	101	G	. . . .	. . . .	. . . .	. . . .
06.O17CC	101	. . . .	. . . .	. . . .	. . . .	. . . .
07.O11P	101	. . . .	. . . .	. . . .	. . . .	. . . .
08.O221NP	101	. . . .	. . . .	. . . .	. . . .	. . . .
09.O222NP	101	. . . .	. . . .	. . . .	. . . .	. . . .
010.O116CP	101	. . . .	. . . .	. . . .	. . . .	. . . .
011.O123MB	101	. . . .	. . . .	. . . .	. . . .	. . . .
012.O212TOK	101	. . . .	. . . .	. . . .	. . . .	. . . .
013.O105MOT	101	. . . .	. . . .	. . . .	. . . .	. . . .
014.O150SI	101	. . . .	. . . .	. . . .	. . . .	. . . .
015.O138FP	101	. . . .	. . . .	. . . .	. . . .	. . . .
016.O174EC	101	. . . .	. . . .	. . . .	. . . .	. . . .
017.O166WE	101	. . . .	. . . .	. . . .	. . . .	. . . .
018.O253HAW	101	. . . .	. . . .	. . . .	. . . .	. . . .
019.O255HAW	101	. . . .	. . . .	. . . .	. . . .	. . . .
020.O235CK	101	. . . .	. . . .	. . . .	. . . .	. . . .
021.O233CK	101	. . . .	. . . .	. . . .	. . . .	. . . .

Table A3.2.1 continued

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01.O2K          151  T G A C T G C T C C   G A G C C A T C C A   C G C T A A T G G G   G C C T C C T G A T   T T T T C A T T T G
02.O73LPB       151  . . . . . . . . . .   . . . . . . C . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
03.O93DH        151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
04.O16CC        151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
05.O18CC        151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
06.O17CC        151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
07.O11P         151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
08.O221NP       151  . . . T . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
09.O222NP       151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
010.O116CP      151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
011.O123MB      151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
012.O212TOK     151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
013.O105MOT     151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
014.O150SI      151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
015.O138FP      151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
016.O174EC      151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
017.O166WE      151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
018.O253HAW     151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
019.O255HAW     151  . . . . . C . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
020.O235CK      151  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .
021.O233CK      151  . . . . . . . . . .   . . . . T . . . . .   . . . . . . . . . .   . . T . . . . . . .   . . . . . . . . . .

01.O2K          201  C A T T T A T C T T   C A T A T T G G A C   G A G G A C T T T A   T T A T G G C T C A   T A C C T T T A C A
02.O73LPB       201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
03.O93DH        201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
04.O16CC        201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
05.O18CC        201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
06.O17CC        201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
07.O11P         201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
08.O221NP       201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
09.O222NP       201  . . . . . . . . . .   . . . . . . . . . T   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
010.O116CP      201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
011.O123MB      201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . T . . . . . . .
012.O212TOK     201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
013.O105MOT     201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
014.O150SI      201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
015.O138FP      201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
016.O174EC      201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
017.O166WE      201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . G .   . . . . . . . . . .   . . . . . . . . . .
018.O253HAW     201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
019.O255HAW     201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
020.O235CK      201  . . . . . . . . . .   . . . . . C . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
021.O233CK      201  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .

01.O2K          251  T C C A C A C C T G   A A A T G T A G G G   G T C A T T T T A T   T A T T A A C T A C   T A T A G G A A C A
02.O73LPB       251  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
03.O93DH        251  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
04.O16CC        251  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . G . . . . . .
05.O18CC        251  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . G . . . . . .
06.O17CC        251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
07.O11P         251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
08.O221NP       251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
09.O222NP       251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
010.O116CP      251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
011.O123MB      251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
012.O212TOK     251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . C . . . . .
013.O105MOT     251  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
014.O150SI      251  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
015.O138FP      251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . T . . .   . . . . . . . . . .
016.O174EC      251  . . . . . . . . . .   . . . . . . T . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
017.O166WE      251  . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
018.O253HAW     251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
019.O255HAW     251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
020.O235CK      251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
021.O233CK      251  . . . . . . . . . .   . . . . . . G . . .   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
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Table A3.2.1 continued

01.O2K	301	G	C	T	T	T	T	T	T	A	G	G	C	T	A	C	G	T	C	T	T	A	C	C	C	T	G	A	G
02.O73LPB	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
03.O93DH	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
04.O16CC	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
05.O18CC	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
06.O17CC	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
07.O11P	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
08.O221NP	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
09.O222NP	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
010.O116CP	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
011.O123MB	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
012.O212TOK	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
013.O105MOT	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
014.O150SI	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
015.O138FP	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
016.O174EC	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
017.O166WE	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
018.O253HAW	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
019.O255HAW	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
020.O235CK	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
021.O233CK	301	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table A3.2.2 Diversity indices plus standard deviation and sample size, for *C. ornata* populations. Fixed populations are not included.

Population	n	π	± s.d.	*h*	± s.d.
CC	10	0.002651	0.00292	0.6444	0.1518
CK	11	0.003677	0.002858	0.6182	0.1643
CP	10	0.000611	0.000929	0.2	0.1541
DH	20	0.001512	0.001504	0.4684	0.1045
EC	10	0.002652	0.002293	0.5333	0.1801
FP	9	0.000679	0.000997	0.2222	0.1662
Haw	10	0.001834	0.001793	0.5333	0.1801
K	10	0.001426	0.001529	0.4667	0.1318
Kap	10	0.000611	0.000929	0.2	0.1541
LPB	10	0.002037	0.001921	0.6	0.1305
MB	5	0.001222	0.001553	0.4	0.2373
Mot	10	0.002241	0.002046	0.6444	0.1012
NP	10	0.001834	0.001793	0.5333	0.1801
Rag	10	0.001426	0.001529	0.4667	0.1318
SI	15	0.000815	0.001057	0.2571	0.1416
Tat	10	0.00245	0.002172	0.3778	0.1813
Tim	9	0.000679	0.000997	0.2222	0.1662
Tok	10	0.002926	0.002454	0.5333	0.1801
We	10	0.000611	0.000929	0.2	0.1541

Table A3.2.3 Haplotype frequencies for 21 *C. ornata* cytochrome *b* haplotypes in all populations sampled.

	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	O11	O12	O13	O14	O15	O16	O17	O18	O19	O20	O21	Total
CC	**0.6**	0	0	**0.2**	**0.1**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
CK	**0.091**	0	0	**0.091**	0	0	**0.636**	0	0	0	0	0	0	0	0	0	0	0	0	**0.091**	**0.091**	0.037
CP	0	0	0	0	0	0	**0.9**	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0.033
DH	**0.7**	0	**0.05**	**0.25**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.067
D	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.037
EC	0	0	0	0	0	0	**0.7**	0	0	**0.1**	0	**0.1**	0	0	0	**0.1**	0	0	0	0	0	0.033
K	**0.7**	0	0	**0.3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
FP	0	0	0	0	0	0	**0.889**	0	0	0	0	0	0	0	**0.111**	0	0	0	0	0	0	0.03
Haw	0	0	0	0	0	**0.1**	**0.7**	0	0	0	0	0	0	0	0	0	0	**0.1**	**0.1**	0	0	0.033
IB	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.03
Kap	0	0	0	0	0	0	**0.9**	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0.033
CR	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
Tok	**0.1**	0	0	0	0	0	**0.7**	0	0	**0.1**	0	**0.1**	0	0	0	0	0	0	0	0	0	0.033
LPB	**0.6**	**0.1**	0	**0.3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
MB	0	0	0	0	0	0	**0.8**	0	0	0	**0.2**	0	0	0	0	0	0	0	0	0	0	0.017
M	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
Mot	**0.5**	0	0	**0.4**	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0.033
NP	0	0	0	0	0	0	**0.7**	**0.1**	**0.1**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0.033
NH	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
Om	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.02
Oh	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
Rag	0	0	0	0	0	0	**0.7**	0	0	**0.3**	0	0	0	0	0	0	0	0	0	0	0	0.033
P	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
SI	**0.867**	0	0	**0.067**	0	0	0	0	0	0	0	0	0	**0.067**	0	0	0	0	0	0	0	0.05
Tat	0.1	0	0	0	0	0	**0.8**	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0.033
TB	0	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
Tim	**0.889**	0	0	**0.111**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.03
WP	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.017
We	**0.9**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0.033
WPB	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.033
Total	0.377	0.003	0.003	0.067	0.003	0.007	0.463	0.003	0.003	0.027	0.003	0.01	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	1

Table A3.2.4. Population differentiation distances (d_{XY} (above diagonal); d_A (below diagonal); d_X (diagonal)) for *C. ornata* populations.

	CC	CK	CP	CR	D	DH	EC	FP	Haw	IB	K	Kap	LPB	M	MB	Mot	NH	NP	Oh	Om	P	Rag	SI	Tat	TB	Tim	Tok	WPB	We	WPB
CC	**1.0**	1.3	5.2	4.8	4.7	4.6	1.2	4.8	1.4	1.0	5.0	1.5	4.6	5.2	1.0	4.6	4.6	5.2	1.1	4.5	4.6	1.1	4.7	1.1	1.0	1.0	4.7	1.1	1.0	1.0
CK	3.8	**4.3**	2.0	1.5	1.7	1.3	4.3	1.5	4.5	3.8	1.7	4.7	1.3	1.9	3.8	1.3	1.3	1.9	4.0	1.8	1.3	4.0	2.0	3.9	3.8	3.8	2.0	3.9	3.8	3.8
CP	4.2	4.8	**0.9**	0.4	0.7	0.2	4.8	0.4	5.0	4.2	0.6	5.2	0.2	0.8	4.2	0.2	0.2	0.7	4.4	0.9	0.2	4.4	1.0	4.3	4.2	4.2	1.0	4.3	4.2	4.2
CR	4.0	4.6	0.8	**0.2**	0.5	0.0	4.6	0.2	4.8	4.0	0.4	5.0	0.0	0.6	4.0	0.0	0.0	0.6	4.2	0.7	0.0	4.2	0.9	4.1	4.0	4.0	0.9	4.1	4.0	4.0
D	0.0	0.6	4.6	4.2	**4.1**	4.0	0.6	4.2	0.8	0.0	4.4	1.0	4.0	4.6	0.0	4.0	4.0	4.6	0.2	3.9	4.0	0.2	4.1	0.1	0.0	0.0	4.1	0.1	0.0	0.0
DH	0.1	1.0	5.2	4.8	4.7	**4.6**	0.9	4.8	1.1	0.6	5.0	1.2	4.6	5.2	0.6	4.6	4.6	5.2	0.7	4.5	4.6	0.7	4.7	0.7	0.6	0.6	4.7	0.7	0.6	0.6
EC	3.9	4.0	1.3	1.0	1.3	0.8	**5.2**	0.9	5.4	4.6	1.2	5.6	0.8	1.3	4.6	0.8	0.8	1.0	4.8	1.3	0.8	4.8	1.4	4.7	4.6	4.6	1.4	4.7	4.6	4.6
FP	4.0	4.1	0.1	0.4	0.7	0.2	4.8	**0.4**	5.0	4.2	0.6	5.2	0.2	0.8	4.2	0.2	0.2	0.8	4.4	0.9	0.2	4.4	1.1	4.3	4.2	4.2	1.1	4.3	4.2	4.2
Haw	3.6	3.7	0.1	0.0	1.0	0.5	4.7	0.7	**4.9**	4.1	0.9	5.1	0.5	1.1	4.1	0.5	0.5	1.1	4.3	1.2	0.5	4.3	1.4	4.2	4.1	4.1	1.4	4.2	4.1	4.1
IB	4.0	4.1	0.1	0.0	0.0	0.0	4.6	0.2	4.8	**4.0**	0.4	5.0	0.0	0.6	4.0	0.0	0.0	0.6	4.2	0.7	0.0	4.2	0.9	4.1	4.0	4.0	0.9	4.1	4.0	4.0
K	0.1	-0.1	4.1	4.1	3.7	4.1	0.9	4.8	1.0	0.6	**5.0**	1.1	4.6	5.2	0.6	4.6	4.6	5.2	0.7	4.5	4.6	0.7	4.7	0.7	0.6	0.6	4.7	0.7	0.6	0.6
Kap	4.0	4.1	0.0	0.0	0.0	0.0	4.1	0.4	5.0	4.2	0.6	**5.2**	0.2	0.8	4.2	0.2	0.2	0.7	4.4	0.9	0.2	4.4	1.0	4.3	4.2	4.2	1.0	4.3	4.2	4.2
LPB	0.1	-0.1	4.1	4.1	3.7	4.1	-0.1	4.1	1.3	0.8	5.2	1.3	**4.8**	5.4	0.8	4.8	4.8	5.4	0.9	4.7	4.8	0.9	4.9	0.9	0.8	0.8	4.9	0.9	0.8	0.8
M	0.0	0.1	3.9	4.0	3.6	4.0	0.1	4.0	0.1	0.0	4.4	1.0	4.0	**4.6**	0.0	4.0	4.0	4.6	0.2	3.9	4.0	0.2	4.1	0.1	0.0	0.0	4.1	0.1	0.0	0.0
MB	4.0	4.1	0.1	0.0	0.0	0.0	4.1	0.0	4.1	4.0	0.8	5.4	0.4	1.0	**4.4**	0.4	0.4	1.0	4.6	1.1	0.4	4.6	1.3	4.5	4.4	4.4	1.3	4.5	4.4	4.4
Mot	0.3	0.0	4.2	4.3	3.9	4.3	-0.1	4.3	-0.1	0.3	4.3	1.5	5.0	5.6	1.0	**5.0**	5.0	5.6	1.1	4.9	5.0	1.0	5.1	1.1	1.0	1.0	5.1	1.1	1.0	1.0
NH	4.0	4.1	0.1	0.0	0.0	0.0	4.1	0.0	4.1	4.0	0.0	4.3	0.0	0.6	4.0	0.0	**0.0**	0.6	4.2	0.7	0.0	4.2	0.9	4.1	4.0	4.0	0.9	4.1	4.0	4.0
NP	4.0	4.1	0.0	0.0	0.0	0.0	4.1	0.0	4.1	4.0	0.0	4.3	0.0	1.2	4.6	0.6	0.6	**1.1**	4.8	1.3	0.6	4.8	1.4	4.7	4.6	4.6	1.4	4.7	4.6	4.6
Oh	0.0	0.1	3.9	4.0	3.6	4.0	0.1	4.0	0.1	0.0	4.0	0.3	4.0	4.0	0.0	4.0	4.0	4.6	**0.2**	3.9	4.0	0.2	4.1	0.1	0.0	0.0	4.1	0.1	0.0	0.0
Om	4.0	4.1	0.1	0.0	0.0	0.0	4.1	0.0	4.1	4.0	0.0	4.3	0.0	0.0	4.0	0.0	0.0	0.6	4.2	**0.7**	0.0	4.2	0.9	4.1	4.0	4.0	0.9	4.1	4.0	4.0
P	4.0	4.1	0.1	0.0	0.0	0.0	4.1	0.0	4.1	4.0	0.0	4.3	0.0	0.0	4.0	0.0	0.0	0.6	4.2	0.7	**0.0**	4.2	0.9	4.1	4.0	4.0	0.9	4.1	4.0	4.0
Rag	4.1	4.2	-0.1	0.1	0.1	0.1	4.3	0.0	4.3	4.1	0.1	4.4	0.1	0.0	4.1	0.1	0.1	0.9	4.8	1.2	0.6	**4.8**	1.3	4.7	4.6	4.6	1.3	4.7	4.6	4.6
SI	0.0	0.0	3.9	4.0	3.6	4.0	0.1	4.0	0.1	0.0	4.0	0.2	4.0	4.0	0.0	4.0	4.0	4.1	0.4	4.1	4.2	0.4	**4.3**	0.3	0.2	0.2	4.3	0.3	0.2	0.2
Tat	3.2	3.3	0.0	0.0	0.0	0.0	3.3	0.0	3.3	3.2	0.0	3.5	0.0	0.0	3.2	0.0	0.0	0.0	3.2	1.4	0.7	4.1	1.4	**4.0**	3.9	3.9	1.4	4.0	3.9	3.9
TB	4.0	4.1	0.1	0.0	0.0	0.0	4.1	0.0	4.1	4.0	0.0	4.3	0.0	0.0	4.0	0.0	0.0	0.1	4.0	0.0	0.0	4.2	0.9	4.1	**4.0**	4.0	0.9	4.1	4.0	4.0
Tim	0.0	0.0	3.9	4.0	3.6	4.0	0.0	4.0	0.0	0.0	4.0	0.1	4.0	4.0	0.0	4.0	4.0	4.1	0.0	3.2	4.0	0.4	4.3	0.3	0.2	**0.2**	4.3	0.3	0.2	0.2
Tok	3.2	3.3	-0.1	0.0	0.0	0.0	3.4	0.0	3.4	3.2	0.0	3.5	0.0	0.0	3.2	0.0	0.0	-0.1	3.2	-0.1	0.0	3.2	1.7	4.2	4.1	4.1	**1.7**	4.2	4.1	4.1
WP	0.0	0.1	3.9	4.0	3.6	4.0	0.1	4.0	0.1	0.0	4.0	0.3	4.0	4.0	0.0	4.0	4.0	4.1	0.0	3.2	4.0	0.0	3.2	0.2	0.1	0.1	3.2	**0.2**	0.1	0.1
We	0.0	0.1	3.9	4.0	3.6	4.0	0.1	4.0	0.1	0.0	4.0	0.3	4.0	4.0	0.0	4.0	4.0	4.1	0.0	3.2	4.0	0.0	3.2	0.0	0.0	0.0	3.2	0.0	**0.0**	0.0
WPB	0.0	0.1	3.9	4.0	3.6	4.0	0.1	4.0	0.1	0.0	4.0	0.3	4.0	4.0	0.0	4.0	4.0	4.1	0.0	3.2	4.0	0.0	3.2	0.0	0.0	0.0	3.2	0.0	0.0	**0.0**

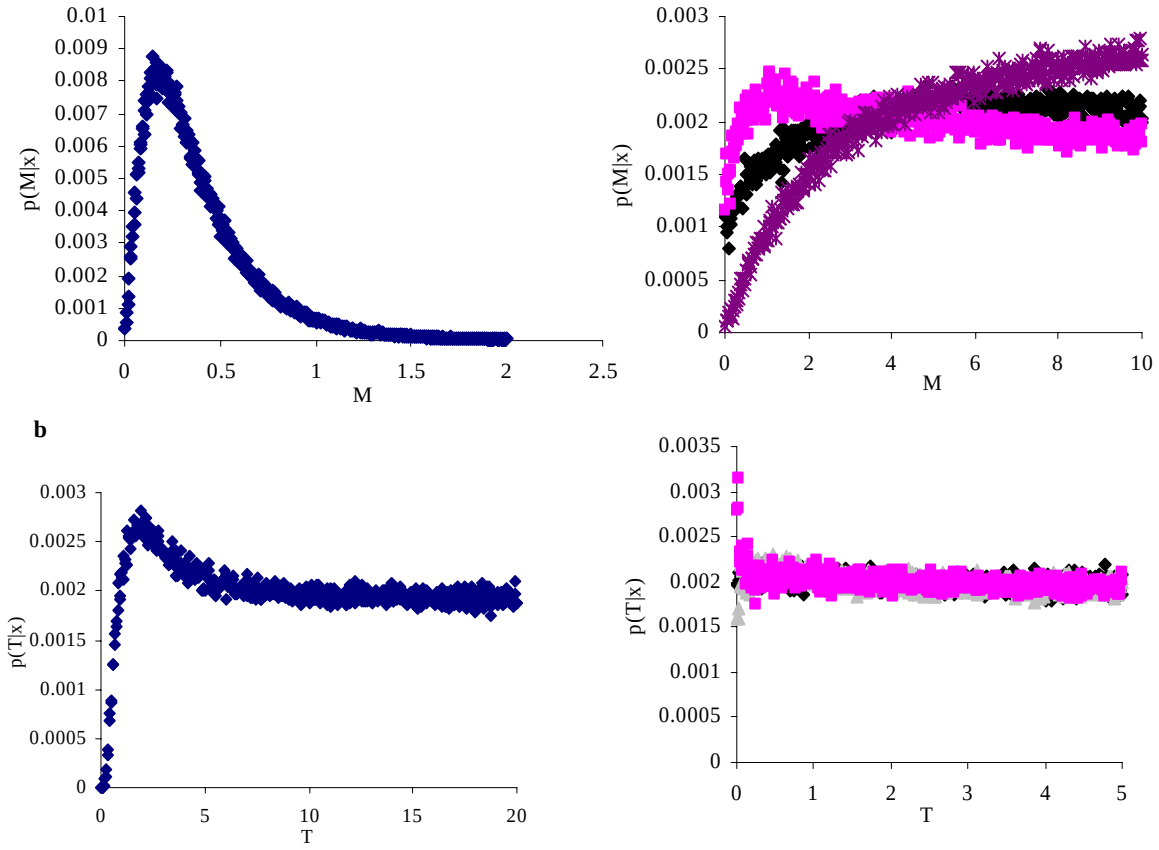

Figure A3.2.1. Posterior distributions of M and T for *C. ornata*. The distrbutions were generated under an infinite-sites model; a Markov chain length of 5 x 10^6 was used with a burn-in time of 5 x 10^5. a) Cook Strait: Mmax = 2 and Tmax = 20; θ = 0.8. b) north east: Mmax = 10 and Tmax = 5;.

Cellana radians

Table A3.2.5 Haplotype alignment for 29 *C. radians* cytochrome *b* haplotypes

```
R1.R1DH          1   CTCAGCATGA TGAAACTTTG GTTCCCTACT AGGTCTTTGC TTAATTATAC
R2.R04CC         1   .......... .......... .......... .......... ..........
R3.R10WPB        1   .......... .......... .......... .......... ..........
R4.ST24TIM       1   .......... .......... .......... .......... ..........
R5.R22M          1   .......... .......... .......... .......... ..........
R6.R27M          1   .......... .......... .......... .......... ..........
R7.R32DH         1   .......... .......... .......... .......... ..........
R8.R41TTP        1   .......... .......... .......... .......... ..........
R9.R46TTP        1   .......... .......... .......... .......... ..........
R10.R47TTP       1   .......... .......... .......... .......... ..........
R11.R55D         1   .......... .......... .........T .......... ..........
R12.R68WP        1   .......... .......... .......... .......... ..........
R13.R87TIM       1   .......... .......... .......... .......... ..........
R14.R103MOT      1   .......... .......... .......... .......... ..........
R15.R155MB       1   .......... .......... .......... .......... ..........
R16.R117MB       1   .......... .......... .......... .......... ..........
R17.R144LPB      1   .......... .......... .......... .......... ..........
R18.ST22TIM      1   .......... .......... .......... .......... ..........
R19.R164IB       1   .......... .......... .......... .......... ..........
R20.ST21TIM      1   .......... .......... .......... .......... ..........
R21.R187TAT      1   .......... .......... .......... .......... ..........
R22.R202OHOP     1   .......... .......... .......... .......... ..........
R23.R245CK       1   .......... .......... .......... .......... .....C....
R24.R251CK       1   .......... .......... .......... .......... ..........
R25.WE5          1   .......... .......... .......... .......... ..........
R26.R262CR       1   .......... .......... .......... .......... ..........
R27.R286JH       1   .......... .......... .......... .......... ..........
R28.R301FP       1   .......... .......... .......... .......... ..........
R29.RST5WPB      1   .......... .......... .......... .......... ..........

R1.R1DH         51   AAATTCTAAC GGGCTTATTT TTATCAATAC ACTACACCGC AAATATTGAT
R2.R04CC        51   .......... .......... .......... .......... ..........
R3.R10WPB       51   .......... .......... .......... .......... ..........
R4.ST24TIM      51   .......... .......... .......... .......... ..........
R5.R22M         51   .......... .......... .......... .......... ..........
R6.R27M         51   .......... .......... .......... .......... ..........
R7.R32DH        51   .......... .......... .......... .......... ..........
R8.R41TTP       51   .......... .......... .......... .......... ..........
R9.R46TTP       51   .......... .......... .......... .......... ..........
R10.R47TTP      51   .......... .......... .......... .......... ..........
R11.R55D        51   .......... .......... .......... .......... ..........
R12.R68WP       51   .......... .......... .......... .......... ..........
R13.R87TIM      51   .......... .......... .......... .......... ..........
R14.R103MOT     51   .......... .......... .......... .......... ..........
R15.R155MB      51   .......... .......... .......... .......... ..........
R16.R117MB      51   .......... .......... .......... .......... ..........
R17.R144LPB     51   .......... .......... .......... .......... ..........
R18.ST22TIM     51   .......... .......... .......... .......... ..........
R19.R164IB      51   .G........ .......... .......... .......... ..........
R20.ST21TIM     51   .......... .......... .......... .......... ..........
R21.R187TAT     51   .......... .......... .......... .......... ..........
R22.R202OHOP    51   ..........T .......... .......... .......... ..........
R23.R245CK      51   .......... .......... .......... .......... ..........
R24.R251CK      51   .......... .......... .......... .......... ..........
R25.WE5         51   .......... .......... .......... .......... ..........
R26.R262CR      51   .......... .......... .......... .......... ..........
R27.R286JH      51   .......... .......... .......... .......... ..........
R28.R301FP      51   .......... .......... .......... .......... ..........
R29.RST5WPB     51   .......... .......... .......... .......... ..........

R1.R1DH        101   ACAGCATTCT CATCTGTAGC TCACATCTCA CGAGATGTAA ATTATGGGTG
R2.R04CC       101   .......... .......... .......... .......... .C........
R3.R10WPB      101   .......... .......... .......... .......... ..........
R4.ST24TIM     101   .......... .......... .......... .......... ..........
R5.R22M        101   .......... .......... .......... .......... ..........
R6.R27M        101   .......... .......... .......... .......... .C........
R7.R32DH       101   .......... .......... .......... .......... ..........
R8.R41TTP      101   .......... .......... .......... .......... ..........
R9.R46TTP      101   .......... .......... .......... .......... ..........
R10.R47TTP     101   .......... .......... .......... .......... ..........
R11.R55D       101   .......... .......... .......... .......... .C........
R12.R68WP      101   .......... .......... .......... .......... ..........
R13.R87TIM     101   .......... .......... .......... .......... ..........
R14.R103MOT    101   .......... .......... .......... .......... ..........
R15.R155MB     101   .......... .......... .......... .......... ..........
R16.R117MB     101   .......... .......... .......... .......... ..........
R17.R144LPB    101   .......... .......... .......... .......... ..........
R18.ST22TIM    101   .......... .......... .......... .......... ..........
R19.R164IB     101   .......... .......... .......... .......... ..........
R20.ST21TIM    101   .......... .......... .......... .......... ..........
```

Table A3.2.5 continued

```
R21.R187TAT   101 ..........  ..........  ..........  ..........  ..........
R22.R202OHOP  101 ..........  ..........  ..........  ..........  ..........
R23.R245CK    101 ..........  ..........  ..........  ..........  ..........
R24.R251CK    101 ..........  ..........  ..........  ..........  .C.....A..
R25.WE5       101 ..........  ..........  ..........  ..........  .....C....
R26.R262CR    101 ..........  ..........  ..........  ..........  ..........
R27.R286JH    101 ..........  ....C.....  ..........  ..........  ..........
R28.R301FP    101 ..........  ..........  ..........  ..........  ..........
R29.RST5WPB   101 ..........  .......G..  ..........  ..........  ..........

R1.R1DH       151 ATTACTACGA  GCTCTTCATG  CAAATGGGGC  CTCTTGGTTT  TTTATCTGTA
R2.R04CC      151 ..........  ..........  ..........  ..........  ..........
R3.R10WPB     151 ..........  ..........  ..........  ..........  ..........
R4.ST24TIM    151 ..........  ..........  ..........  ..........  ..........
R5.R22M       151 ..........  ..........  ..........  ..........  ..........
R6.R27M       151 ..........  ..........  ..........  ..........  ..........
R7.R32DH      151 ..........  ..........  ..........  ..........  ..........
R8.R41TTP     151 ..........  ..........  ..........  ..........  ..........
R9.R46TTP     151 ....T.....  ..........  ..........  ..........  ..........
R10.R47TTP    151 ..........  ..........  ..........  ..........  ..........
R11.R55D      151 ..........  ..........  ..........  ..........  ..........
R12.R68WP     151 ..........  ..........  .......A..  ..........  ..........
R13.R87TIM    151 .C........  ..........  ..........  ..........  ..........
R14.R103MOT   151 ..........  ..........  ..........  ..........  ..........
R15.R155MB    151 G.........  ..........  ..........  ..........  ..........
R16.R117MB    151 ..........  ..........  ..........  ..........  ..........
R17.R144LPB   151 ..........  ..........  ..........  ..........  ..........
R18.ST22TIM   151 ..........  ..........  ..........  ..........  ..........
R19.R164IB    151 ..........  ..........  ..........  ..........  ..........
R20.ST21TIM   151 ..........  ..........  ..........  ..........  ..........
R21.R187TAT   151 ..........  ..........  ..........  ..........  ..........
R22.R202OHOP  151 ..........  ..........  ..........  ..........  ..........
R23.R245CK    151 ..........  ..........  ..........  ..........  ..........
R24.R251CK    151 ..........  ..........  ..........  ..........  ..........
R25.WE5       151 ..........  ..........  ..........  ..........  ..........
R26.R262CR    151 ..........  ..........  ..........  ..........  ..........
R27.R286JH    151 ..........  ..........  ..........  ..........  ..........
R28.R301FP    151 ..........  ..........  ..........  ..........  ..........
R29.RST5WPB   151 ..........  ..........  ..........  ..........  ..........

R1.R1DH       201 TCTACTTACA  CATTGGCCGA  GGAATTTACT  ACGGCTCATA  TCTTTATATT
R2.R04CC      201 ..........  ..........  ..........  ..........  ..........
R3.R10WPB     201 .T........  ..........  ..........  ..........  ..........
R4.ST24TIM    201 ..........  ..........  ..........  ..........  ..........
R5.R22M       201 ..........  ..........  ..........  ..........  C.........
R6.R27M       201 ..........  ..........  ..........  ..........  ..........
R7.R32DH      201 .......G..  ..........  ..........  ..........  ..........
R8.R41TTP     201 ..........  ..........  ..........  ..........  ..........
R9.R46TTP     201 ..........  ..........  ..........  ..........  ..........
R10.R47TTP    201 ..........  ..........  ..........  ..........  ..........
R11.R55D      201 ..........  ..........  ..........  ..........  ..........
R12.R68WP     201 ..........  ..........  ..........  ..........  ..........
R13.R87TIM    201 .T........  ..........  ..........  ..........  ..........
R14.R103MOT   201 .T........  ..........  ..........  ..........  ..........
R15.R155MB    201 ..........  ..........  ..........  ..........  ..........
R16.R117MB    201 ..........  ..........  ..........  ..........  ..........
R17.R144LPB   201 .T........  ..........  ..........  ..........  ..........
R18.ST22TIM   201 ..........  ..........  ..........  ..........  ..........
R19.R164IB    201 ..........  ..........  ..........  ..........  ..........
R20.ST21TIM   201 .T.....G..  ..........  ..........  ..........  ..........
R21.R187TAT   201 ..........  ..........  ..........  ..........  ..........
R22.R202OHOP  201 ..........  ..........  ..........  ..........  ..........
R23.R245CK    201 ..........  ..........  ..........  ..........  ..........
R24.R251CK    201 ..........  ..........  ..........  ..........  .......G..
R25.WE5       201 .T........  ..........  ..........  ..........  ..........
R26.R262CR    201 ..........  ...c......  ..........  ..........  ..........
R27.R286JH    201 ..........  ..........  ..........  ..........  ..........
R28.R301FP    201 ..........  ..........  ..........  ..........  .......G..
R29.RST5WPB   201 .T........  ..........  ..........  ..........  ..........

R1.R1DH       251 CACACGTGGA  ACGTAGGGGT  CATCTTATTA  TTAACAACTA  TAGGAACGGC
R2.R04CC      251 ..........  ..........  ..........  ..........  ..........
R3.R10WPB     251 ..........  ..........  ..........  C.........  ..........
R4.ST24TIM    251 ..........  ..........  ..........  ..........  ..........
R5.R22M       251 ..........  ..........  ..........  ..........  ..........
R6.R27M       251 ..........  ..........  ..........  ..........  ..........
R7.R32DH      251 ..........  ..........  ..........  ..........  ..........
R8.R41TTP     251 ..........  ..........  ..........  .........C  ..........
R9.R46TTP     251 ..........  ..........  ..........  ..........  ..........
R10.R47TTP    251 .........A  ..........  ..........  ..........  ..........
```

Table A3.2.5 continued

R11.R55D	251	. . . .	. . . .	. . . .	. . . .	. . . .
R12.R68WP	251	. . . .	. . . .	. . . .	. . . .	. . . .
R13.R87TIM	251	. . . .	. . . .	. . . .	C	. . . .
R14.R103MOT	251	. . . .	. . . . G	. . . .	C	. . . .
R15.R155MB	251	. . . .	. . . .	. . . .	. . . .	. . . .
R16.R117MB	251	. . . .	. . . .	. . . .	. . . .	. . . .
R17.R144LPB	251	. . . .	. . . .	. . . .	. . . .	. . . .
R18.ST22TIM	251	. . . .	. . . .	. . . .	c	. . . .
R19.R164IB	251	. . . .	. . . .	. . . .	. . . .	. . . .
R20.ST21TIM	251	. . . .	. . . .	. . . .	C	. . . .
R21.R187TAT	251	. . . . A .	. . . .	. . . .	. . . .	. . . .
R22.R202OHOP	251	. . . .	. . . .	. . . .	. . . .	. . . .
R23.R245CK	251	. . . .	. . . .	. . . .	. . . .	. . . .
R24.R251CK	251	. . . .	. . . .	. . . .	. . . .	. . . .
R25.WE5	251	. . . .	. . . .	. . . .	. . . .	. . . .
R26.R262CR	251	. . . .	. . . .	. . . .	. . . .	. . . .
R27.R286JH	251	. . . .	. . . .	. . . .	. . . .	. . . .
R28.R301FP	251	. . . .	. . . .	. . . .	. . . .	. . . .
R29.RST5WPB	251	. . . .	. . . .	. . . .	C	. . . .

R1.R1DH	301	C T T C C T G G G C	T A C G T C T T A C	C C T G A G
R2.R04CC	301	. . . .	. . . .	. . . . . .
R3.R10WPB	301	. . . .	. . . .	. . . . . .
R4.ST24TIM	301	. . . T	. . . .	. . . . . .
R5.R22M	301	. . . .	. . . .	. . . . . .
R6.R27M	301	. . . .	. . . .	. . . . . .
R7.R32DH	301	. . . .	. . . .	. . . . . .
R8.R41TTP	301	. . . .	. . . .	. . . . . .
R9.R46TTP	301	. . . .	. . . .	. . . . . .
R10.R47TTP	301	. . . .	. . . .	. . . . . .
R11.R55D	301	. . . .	. . . .	. . . . . .
R12.R68WP	301	. . . .	. . . .	. . . . . .
R13.R87TIM	301	. . . .	. . . .	. . . . . .
R14.R103MOT	301	. . . .	. . . .	. . . . . .
R15.R155MB	301	. . . .	. . . .	. . . . . .
R16.R117MB	301	. . . . . . A . . .	. . . .	. . . . . .
R17.R144LPB	301	. . . .	. . . .	. . . . . .
R18.ST22TIM	301	. . . .	. . . .	. . . . . .
R19.R164IB	301	. . . .	. . . .	. . . . . .
R20.ST21TIM	301	. . . .	. . . .	. . . . . .
R21.R187TAT	301	. . . . . . A . . .	. . . .	. . . . . .
R22.R202OHOP	301	. . . .	. . . .	. . . . . .
R23.R245CK	301	. . . . . . A . . .	. . . .	. . . . . .
R24.R251CK	301	. . . .	. . . .	. . . . . .
R25.WE5	301	. . . .	. . . .	. . . . . .
R26.R262CR	301	. . . .	. . . .	. . . . . .
R27.R286JH	301	. . . .	. . . .	. . . . . .
R28.R301FP	301	. . . .	. . . .	. . . . . .
R29.RST5WPB	301	. . . .	. . . .	. . . . . .

Table A3.2.6 Diversity indices plus standard deviation and sample size, for *C. radians* populations. Fixed populations are not included.

Population	n	π	± s.d.	h	± s.d.
CC	10	0.00109	0.0013	0.3556	0.1591
CK	14	0.00288	0.00236	0.4945	0.1506
CP	20	0.00031	0.00061	0.1	0.088
CR	10	0.00062	0.00094	0.2	0.1541
D	9	0.00137	0.00151	0.2222	0.1662
DH	13	0.00229	0.00203	0.5256	0.1527
FP	14	0.00132	0.00142	0.3956	0.1588
Haw	5	0.00103	0.00135	0.3333	0.2152
IB	10	0.00171	0.00172	0.5111	0.1643
JH	6	0.00267	0.00249	0.7333	0.1552
K	4	0.00154	0.0019	0.5	0.2652
Kap	7	0.00132	0.0015	0.4286	0.1687
LPB	13	0.00142	0.00149	0.2949	0.1558
M	10	0.0039	0.00302	0.7556	0.1295
MB	10	0.00171	0.00172	0.5111	0.1643
Mot	9	0.00377	0.00298	0.5833	0.1833
NH	10	0.00062	0.00094	0.2	0.1541
Oh	10	0.00123	0.0014	0.3778	0.1813
Om	8	0.00319	0.00269	0.6786	0.122
Rag	10	0.00062	0.00094	0.2	0.1541
SI	24	0.001	0.00117	0.2391	0.1129
Tat	12	0.00177	0.00173	0.4394	0.1581
Tim	11	0.00493	0.00356	0.8	0.1138
Tok	10	0.00062	0.00094	0.2	0.1541
TTP	9	0.00205	0.00196	0.5833	0.1833
We	10	0.00294	0.00247	0.6444	0.1518
WP	4	0.00555	0.00445	0.8	0.164
WPB	11	0.00505	0.00362	0.7818	0.0926

Table A3.2.7 Haplotype frequencies for 29 *C. radians* cytochrome *b* haplotypes in all populations sampled.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Total
CC	**0.8**	**0.2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CK	**0.7**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	**0.1**	0	0	0	0	0	0
CR	**0.9**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0
CP	**1**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1
D	**1.6**	0	0	0	0	0	0	0	0	0	**0.2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DH	**0.7**	0	0	0	**0.2**	**0.1**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EC	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FP	**0.8**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0
Haw	**0.8**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IB	**0.7**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.2**	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0
JH	**0.5**	0	0	0	**0.3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.2**	0	0	0
K	**0.8**	0	0	0	**0.3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kap	**0.9**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LPB	**0.8**	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0
M	**0.5**	**0.2**	**0.1**	0	**0.1**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MB	**0.7**	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	**0.2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mot	**0.7**	0	**0.1**	0	0	0	0	0	0	0	0	**0.1**	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NP	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NH	**0.9**	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Om	**0.5**	0	**0.1**	0	**0.4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oh	**0.8**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0
P	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rag	**0.9**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SI	**0.9**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1
Tat	**0.8**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.2**	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0
Tim	**0.5**	0	**0.2**	**0.1**	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	**0.1**	0	**0.1**	0	0	0	0	0	0	0	0	0	0
Tok	**0.9**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TTP	**0.7**	0	0	0	0	0	0	**0.1**	**0.1**	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WP	**0.3**	0	**0.5**	0	0	0	0	0	0	0	0	**0.3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
We	**0.6**	**0.2**	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0
WPB	**0.4**	0	**0.4**	0	**0.1**	0	0	0	0	0	0	0	**0.1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.1**	0
Total	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table A3.2.8. Population differentiation distances (d_{XY} (above diagonal); d_A (below diagonal); d_X (diagonal)) for *C. radians* populations.

	CC	CK	CP	CR	D	DH	EC	FP	Haw	IB	JH	K	Kap	LPB	M	MB	Mot	NH	NP	Oh	Om	P	Rag	SI	Tat	Tim	Tok	TTP	We	WP	WPB
CC	**0.4**	0.2	0.6	0.3	0.4	0.6	0.2	0.4	0.4	0.5	0.7	0.5	0.5	0.4	0.8	0.5	0.9	0.3	0.2	0.4	0.8	0.2	0.3	0.3	0.5	1.3	0.3	0.5	0.6	1.4	1.6
CK	0.0	**0.1**	0.5	0.2	0.3	0.4	0.1	0.3	0.2	0.4	0.6	0.3	0.3	0.3	0.7	0.4	0.7	0.2	0.1	0.3	0.7	0.1	0.2	0.2	0.4	1.1	0.1	0.4	0.5	1.3	1.4
CP	0.0	0.0	**0.9**	0.6	0.7	0.9	0.5	0.7	0.6	0.8	1.0	0.8	0.8	0.7	1.1	0.8	1.2	0.6	0.5	0.7	1.1	0.5	0.6	0.6	0.8	1.6	0.6	0.8	0.9	1.7	1.9
CR	0.0	0.0	0.0	**0.2**	0.3	0.5	0.1	0.3	0.3	0.4	0.6	0.4	0.4	0.3	0.8	0.4	0.8	0.2	0.1	0.3	0.7	0.1	0.2	0.3	0.4	1.2	0.2	0.4	0.6	1.3	1.5
D	0.0	0.0	0.0	0.0	**0.4**	0.6	0.2	0.4	0.4	0.5	0.7	0.5	0.5	0.4	0.9	0.5	0.9	0.3	0.2	0.4	0.9	0.2	0.3	0.4	0.6	1.3	0.3	0.6	0.7	1.4	1.6
DH	0.0	0.0	0.0	0.0	0.0	**0.7**	0.4	0.6	0.6	0.7	0.8	0.6	0.6	0.6	1.0	0.7	1.1	0.5	0.4	0.6	0.9	0.4	0.5	0.5	0.7	1.5	0.5	0.7	0.8	1.6	1.7
EC	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	0.2	0.2	0.3	0.5	0.3	0.3	0.2	0.7	0.3	0.7	0.1	0.0	0.2	0.6	0.0	0.1	0.2	0.3	1.1	0.1	0.3	0.5	1.2	1.4
FP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.4**	0.4	0.5	0.7	0.5	0.5	0.4	0.9	0.5	0.9	0.3	0.2	0.4	0.8	0.2	0.3	0.4	0.5	1.3	0.3	0.5	0.7	1.4	1.6
Haw	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.3**	0.4	0.7	0.4	0.4	0.4	0.9	0.4	0.8	0.3	0.2	0.3	0.8	0.2	0.2	0.3	0.4	1.3	0.3	0.5	0.7	1.4	1.5
IB	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.6**	0.8	0.6	0.6	0.5	1.0	0.5	1.0	0.4	0.3	0.5	0.9	0.3	0.4	0.5	0.5	1.4	0.4	0.6	0.8	1.5	1.7
JH	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	**0.9**	0.6	0.8	0.7	1.1	0.8	1.2	0.6	0.5	0.7	0.9	0.5	0.6	0.6	0.8	1.6	0.6	0.8	0.9	1.7	1.8
K	0.0	0.0	0.0	0.0	0.0	-0.1	0.0	0.0	0.0	0.0	-0.1	**0.5**	0.5	0.5	0.9	0.6	0.9	0.4	0.3	0.5	0.7	0.3	0.4	0.4	0.6	1.3	0.4	0.6	0.7	1.5	1.6
Kap	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	**0.4**	0.5	1.0	0.6	0.9	0.4	0.3	0.5	0.9	0.3	0.4	0.4	0.6	1.3	0.4	0.6	0.8	1.5	1.6
LPB	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	**0.5**	0.9	0.5	0.9	0.3	0.2	0.4	0.8	0.2	0.3	0.4	0.6	1.3	0.3	0.6	0.7	1.4	1.5
M	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.0	**1.3**	1.0	1.3	0.8	0.7	0.9	1.2	0.7	0.8	0.8	1.0	1.6	0.7	1.0	1.0	1.7	1.8
MB	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	**0.6**	1.0	0.4	0.3	0.5	0.9	0.3	0.4	0.5	0.5	1.4	0.4	0.6	0.8	1.5	1.7
Mot	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1	**1.2**	0.8	0.7	0.9	1.2	0.7	0.8	0.8	1.0	1.4	0.8	1.0	1.1	1.4	1.6
NH	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	**0.2**	0.1	0.3	0.7	0.1	0.2	0.3	0.4	1.2	0.2	0.4	0.6	1.3	1.5
NP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	**0.0**	0.2	0.6	0.0	0.1	0.2	0.3	1.1	0.1	0.3	0.5	1.2	1.4
Oh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	**0.4**	0.8	0.2	0.3	0.4	0.5	1.3	0.3	0.5	0.7	1.4	1.6
Om	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	-0.1	-0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	**1.0**	0.6	0.7	0.8	1.0	1.5	0.7	1.0	1.0	1.6	1.7
P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	**0.0**	0.1	0.2	0.3	1.1	0.1	0.3	0.5	1.2	1.4
Rag	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	**0.2**	0.3	0.4	1.2	0.2	0.4	0.6	1.3	1.5
SI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	**0.3**	0.5	1.3	0.3	0.5	0.6	1.4	1.5
Tat	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	**0.6**	1.4	0.4	0.7	0.8	1.5	1.7
Tim	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.2	0.2	0.3	0.0	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	**1.6**	1.2	1.4	1.5	1.6	1.6
Tok	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.3	**0.2**	0.4	0.6	1.3	1.5
TTP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.3	0.0	**0.7**	0.8	1.5	1.7
We	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	-0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	**1.0**	1.6	1.7
WP	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.2	0.2	0.3	-0.1	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	-0.1	0.3	0.3	0.2	**1.8**	1.7
WPB	0.6	0.5	0.6	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.6	0.5	0.6	0.5	0.4	0.6	0.1	0.5	0.5	0.5	0.3	0.5	0.5	0.5	0.6	-0.1	0.5	0.5	0.4	0.0	**1.6**

Table A3.2.9. Mismatch parameters for *C. radians* populations.
Tau, theta, Sum of squared deviations and the raggedness index are given.

	T	θ1	θ2	SSD	Raggedness
CC	3.162	0	0.635	0.1268	0.6681
CK	6.031	0.006	1.189	0.1	0.4219
CP	3	0.049	0.065	0.0141	0.83
CR	3	0.031	0.18	0.0515	0.72
D	3	0.102	0.156	0.0707	0.7037
DH	4.375	0.008	1.478	0.1205	0.5074
FP	3.367	0	0.759	0.1296	0.6305
Haw	3.188	0.008	0.537	0.1164	0.6667
IB	2.824	0	1.449	0.1749	0.6785
JH	2.367	0	8.105	0.228	0.8267*
K	2.69	0.003	1.685	0.1893	0.75
Kap	2.945	0	1.055	0.1589	0.6939
LPB	3	0	0.305	0.0652	0.5771
M	3.68	0.002	4.921	0.1308	0.5042 *
MB	2.824	0	1.449	0.1749	0.6785
Mot	6.555	0.008	2.214	0.1073	0.3819
NH	3	0.031	0.18	0.0515	0.72
Oh	2.787	0.005	0.637	0.0112	0.2222
Om	2.989	0.566	3.035	0.1427	0.5344
Rag	3	0.031	0.18	0.0515	0.72
SI	3	0.219	0.219	0.0482	0.6402
Tim	4.989	0.011	6.219	0.1132	0.4367 *
TTP	2.733	0	2.134	0.1847	0.6875
Tat	4.328	0.001	0.942	0.1126	0.5351
Tok	3	0.031	0.18	0.0515	0.72
We	3.064	0.001	2.905	0.106	0.3704
WP	6.271	0.002	12.061	0.1455	0.52
WPB	5.145	0.002	6.67	0.1142	0.4317

Note: * - represents populations that do not support the rapid expansion hypotheses as the raggedness index is significantly different ($p < 0.05$)

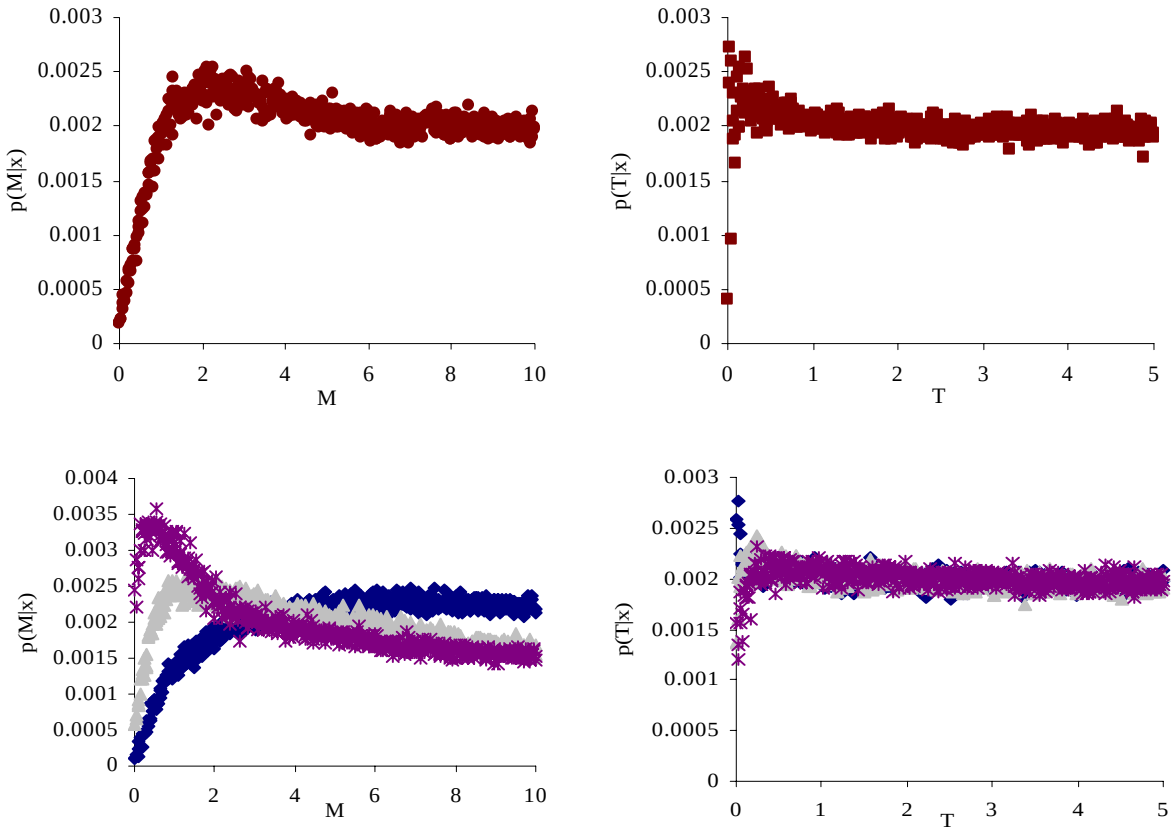

Figure A3.2.2. Posterior distributions of M and T for *C. radians*. The distributions were generated under an infinite-sites model; a Markov chain length of 5 x 10^6 was used with a burn-in time of 5 x 10^5. a) Cook Strait: Mmax = 10 and Tmax = 5; θ = 0.8. b) north east: Mmax = 10 and Tmax = 5; Blue line, CP-Tat; Pink line, CP-CK; Maroon, CK-Tat.

Cellana flava

Table A3.2.10 Haplotype alignment for 21 C. ornata cytochrome *b* haplotypes.

```
F1.F1CC     1   TCCATCCAAC ATCTCAGCAT GATGAAACTT TGGTTCTCTG TTAGGCCTTT
F2.F33EC    1   .......... .......... .......... .......... .....T....
F3.F44TAT   1   .......... .......... .......... .......... ..........
F4.F93CC    1   .......... .......... .......... .......... ..........

F1.F1CC    51   GCTTAATCAT ACAAATTCTA ACTGGCTTAT TTTTATCAAT ACATTACACC
F2.F33EC   51   .......... .......... .......... .......... ..........
F3.F44TAT  51   .......... .......... .......... .......... ..........
F4.F93CC   51   ......C... .......... .......... .......... ..........

F1.F1CC   101   GCGAATATCG ATACAGCATT CTCATCTGTA GCTCACATTT CACGAGATGT
F2.F33EC  101   .......... .......... .......... .......... ..........
F3.F44TAT 101   .......... .......... .......... ..C....... ..........
F4.F93CC  101   .......... .......... .......... .......... ..........

F1.F1CC   151   AAATTATGGG TGACTATTAC GAGCCCTTCA TGCAAATGGG GCCTCTTGGT
F2.F33EC  151   .......... .......... .......... .......... ..........
F3.F44TAT 151   .......... .......... .......... .......... ..........
F4.F93CC  151   .......... .......... .......... .......... ..........

F1.F1CC   201   TTTTTATCTG TATCTACCTA CATATTGGCC GAGGAATTTA CTATGGCTCA
F2.F33EC  201   .......... .......... .......... .......... ..........
F3.F44TAT 201   .......... .......... .......... .......... ..........
F4.F93CC  201   .......... .......... .......... .......... ..........

F1.F1CC   251   TATCTTTATG TTCATACGTG AAACGTCGGA GTTATCTTAT TGTTAACAAC
F2.F33EC  251   .......... .......... .......... .......... ..........
F3.F44TAT 251   .......... .......... .......... .......... ..........
F4.F93CC  251   .......... .......... .......... .......... ..........

F1.F1CC   301   CATAGGAACG GCTTTTCTAG GTTATGTTCT ACCCTGAgGA CAAATATCAT
F2.F33EC  301   .......... .......... .......... .......... ..........
F3.F44TAT 301   .......... .......... .......... .......... ..........
F4.F93CC  301   .......... .......... .......... .......... ..........

F1.F1CC   351   TCTGAGGGG
F2.F33EC  351   .........
F3.F44TAT 351   .........
F4.F93CC  351   .........
```

Table A3.2.11 Diversity indices plus standard deviation and sample size, for *C. ornata* populations. Fixed populations are not included.

2. Diversity Indices

	n	h	± s.d.	π	± s.d.
CC	11	0.1818	0.1436	0.00051	0.0008
CK	10	0.3556	0.1591	0.00099	0.00118
CR	8	0.4286	0.1687	0.0012	0.00136
EC	20	0.6889	0.1038	0.0023	0.00202
Tat	9	0.5000	0.1283	0.0014	0.00148
Tok	10	0.3556	0.1591	0.00099	0.00118

Table A3.2.12 Haplotype frequencies for 4 *C. flava* cytochrome *b* haplotypes in all populations sampled.

	F1	F3	F2	F4	Total
CC	0.91	0.00	0.00	0.09	0.13
CK	0.80	0.20	0.00	0.00	0.12
CR	0.75	0.25	0.00	0.00	0.09
EC	0.60	0.30	0.10	0.00	0.24
K	1.00	0.00	0.00	0.00	0.11
Mot	1.00	0.00	0.00	0.00	0.09
Tat	0.67	0.33	0.00	0.00	0.11
Tok	0.80	0.20	0.00	0.00	0.12
Total	0.79	0.18	0.02	0.01	1.00

Table A3.2.13. Population differentiation distances (d_{XY} (above diagonal); d_A (below diagonal); d_X (diagonal)) for *C. flava* populations.

	K	CC	Mot	CK	Ech	Ecb	Tat	Tok	CR
K	**0.0**	0.1	0.0	0.2	0.5	0.3	0.3	0.2	0.3
CC	0.0	**0.2**	0.1	0.3	0.6	0.4	0.4	0.3	0.3
Mot	0.0	0.0	**0.0**	0.2	0.5	0.3	0.3	0.2	0.3
CK	0.0	0.0	0.0	**0.4**	0.6	0.4	0.4	0.3	0.4
Ech	0.1	0.1	0.1	0.0	**0.8**	0.6	0.6	0.6	0.6
Ecb	0.1	0.1	0.1	0.0	0.0	**0.5**	0.4	0.4	0.4
Tat	0.1	0.1	0.1	0.0	0.0	-	**0.5**	0.4	0.4
Tok	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.4**	0.4
CR	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**0.4**

Table A3.2.14. Haplotype identification, showing the reference specimen and GenBank accession numbers for the cytochrome b gene.

Haplotype. Reference	GenBank accession
Cellana ornata	
01.O2K	DQ011447
02.O73LPB	DQ011448
03.O93DH	DQ011449
04.O16CC	DQ011450
05.O18CC	DQ011451
06.O17CC	DQ011452
07.O11P	DQ011453
08.O221NP	DQ011454
09.O222NP	DQ011455
010.O116CP	DQ011456
011.O123MB	DQ011457
012.O212TOK	DQ011458
013.O105MOT	DQ011459
014.O150SI	DQ011460
015.O138FP	DQ011461
016.O174EC	DQ011462
017.O166WE	DQ011463
018.O253HAW	DQ011464
019.O255HAW	DQ011465
020.O235CK	DQ011466
021.O233CK	DQ011467
Cellana radians	
R1.R1DH	DQ011468
R2.R04CC	DQ011469
R3.R10WPB	DQ011470
R4.ST24TIM	DQ011471
R5.R22M	DQ011472
R6.R27M	DQ011473
R7.R32DH	DQ011474
R8.R41TTP	DQ011475
R9.R46TTP	DQ011476
R10.R47TTP	DQ011477
R11.R55D	DQ011478
R12.R68WP	DQ011479
R13.R87TIM	DQ011480
R14.R103MOT	DQ011481
R15.R155MB	DQ011482
R16.R117MB	DQ011483
R17.R144LPB	DQ011484
R18.ST22TIM	DQ011485
R19.R164IB	DQ011486
R20.ST21TIM	DQ011487
R21.R187TAT	DQ011488
R22.R202OHOP	DQ011489
R23.R245CK	DQ011490
R24.R251CK	DQ011491
R25.WE5	DQ011492
R26.R262CR	DQ011493
R27.R286JH	DQ011494
R28.R301FP	DQ011495
R29.RST5WPB	DQ011496

Haplotype. Reference	GenBank accession
Cellana flava	
F1.F1CC	DQ011497
F2.F33EC	DQ011498
F3.F44TAT	DQ011499
F4.F93CC	DQ011500